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STRUCTURE AND FUNCTION OF THE EGG SHELL AS RELATED TO WATER ABSORPTION BY THE EGGS OF ACHETA DOMESTICUS (L.)[1]

J. E. MCFARLANE

Abstract

The shell of the newly laid egg of *Acheta domesticus* (L.) consists of the chorion, in which two layers can be distinguished: an outer exochorion, about 2.5 μ thick; and an inner endochorion, about 0.4 μ thick, which contains lipoid and a tyrosinase. At about the time water absorption begins, the endochorion breaks up, in a more or less regular way, into many small fragments; as a result, spaces are created in the endochorion, and it seems probable that it is this structural change which permits water to be absorbed by the egg. The breaking up of the endochorion appears to be due to phenolic tanning. Also at about the time water absorption begins, the newly formed serosa begins to lay down the serosal cuticle, first an outer lipoid layer, about 0.4 μ thick, which contains a tyrosinase; and then an inner layer, which is laid down continuously while the serosa exists, and which reaches, at the time water absorption ends, a maximum thickness of 8–10 μ. Thereafter the inner layer of the serosal cuticle is steadily resorbed up to the time the egg is hatched, and the vacated shell consists only of the chorion and the lipoid layer of the serosal cuticle. Water absorption appears to be brought to an end by the phenolic tanning of the lipoid layer of the serosal cuticle.

Introduction

Various studies on the mechanism of water absorption by insect eggs have recently appeared (see refs. 2, 8, 9), but as yet no completely satisfactory explanation of the process has been advanced for any single species. In this article, evidence is presented for the view, suggested in part before (10), that water absorption by the eggs of *Acheta domesticus* (L.) begins and ends with the successive phenolic tanning of two thin lipoprotein layers of the shell, tanning of the first permitting water to enter by increasing the permeability of the shell, and tanning of the second stopping water entry by decreasing the permeability and increasing the rigidity of the shell. Given a shell permeable to water, actual water entry can be accounted for simply by osmosis, and no 'active' mechanism, other than the intact living egg, need be postulated.

Materials and Methods

Methods for handling and incubating cricket eggs have been previously described (9, 10). In this work, the eggs were routinely obtained and handled as follows: egg dishes containing moist sand were placed in the adult cultures (maintained at 28° C) for 12 hours; for the following 12 hours the eggs were

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kept at room temperature, and during this interval the eggs were washed and divided into lots of 50 eggs each, and each lot was placed on moist filter paper in a 2-oz, airtight ointment jar; finally, 24 hours after the egg dishes had been placed in the adult cultures, the jars containing the eggs were placed in a water bath at $33 \pm 0.5°$ C. Timing of the eggs began with incubation in the water bath; thus a 12-hour egg as referred to in this paper is one which, following the above preliminary treatment, had been incubated in the water bath for 12 hours.

In the immersion experiments, the eggs were immersed in 1 ml of the solution under test in a 32-ml shell vial, diameter 25 mm. The vial was stoppered with a rubber stopper and placed in a water bath at $33 \pm 0.5°$ C. The period of treatment varied with the experiment, ranging from 2 to 24 hours. After treatment the eggs were washed with at least two changes of distilled water, and then incubated under optimal conditions (on moist filter paper in an airtight jar at $35 \pm 1°$ C) to determine the percentage survival.

In all immersion experiments, two lots of 50 eggs each were used per test, and many of the tests were repeated. Two lots of control eggs were immersed in distilled water in each experiment, and in those experiments in which eggs were immersed in one solution followed by immersion in another, the appropriate additional controls were run.

For paraffin sections, the eggs were pricked and fixed in Smith's formol-bichromate, impregnated by Peterfi's celloidin–paraffin method, sectioned at 10 μ, and stained with Mallory's triple stain and with Delafield's haematoxylin (11). For frozen sections, egg shells and pricked eggs were fixed in Baker's formaldehyde-calcium, embedded in gelatin, sectioned at 15 μ, and stained with Sudan IV and with Sudan black B (11). Whole egg shells were stained with Grenacher's alum-carmine and mounted in balsam.

All chemicals used were obtained from the Fisher Scientific Co., Montreal, except for the sodium hypochlorite solution, which was the commercial Javex, available chlorine 7%.

Observations were made and experiments routinely carried out on eggs at 24-hour intervals, i.e., at 0, 24, 48, 72, 96, 120, 144, 168, and 192 hours. Where eggs "of any age" are referred to in the text, eggs of precisely 0, 24, 48, etc. hours are meant. Where eggs "48 or more hours old", "60 or more hours old", and "72 or more hours old", etc. are referred to in the text, eggs of precisely 48, 72, 96, etc. hours, 60, 72, 96, etc. hours, and 72, 96, etc. hours are meant. Elsewhere the age of eggs is specified.

Observations

The shell of the newly laid egg consists of the chorion, to which is added, during the period of water absorption, the serosal cuticle (10). The chorion persists throughout embryonic development and forms the larger part of the vacated shell (Fig. 1), whereas the serosal cuticle, after water absorption, is steadily removed from the shell, until, by the time of hatching of the egg, nothing remains of it but a very thin outer layer.

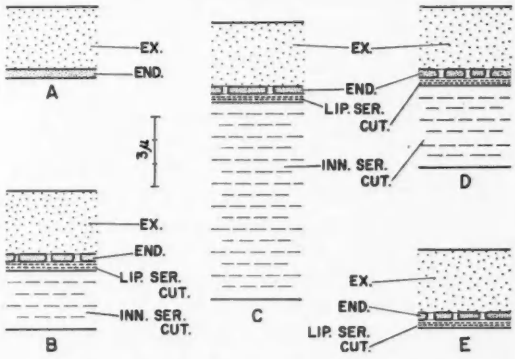

FIG. 1. Cross-section of the shell of the egg of *Acheta domesticus* (L.) at various ages when incubated at 33° C. A, 0 hours; B, 48 hours; C, 96 hours; D, 144 hours; E, vacated shell. *end.*, endochorion; *ex.*, exochorion; *inn. ser. cut.*, inner layer of serosal cuticle; *lip. ser. cut.*, lipoid layer of serosal cuticle.

The Chorion

The structure of the chorion does not change during the first 36 hours of incubation, i. e., before water absorption begins. With Mallory's stain, two layers are well defined:* an outer straw-colored layer about 2.5 μ in thickness and here termed the 'exochorion';† and an inner layer about 0.4 μ in thickness, which stains red and is here termed the 'endochorion'.† The endochorion stains lightly with Sudan IV and Sudan black B. It is not at this time as brittle as the exochorion, and in sections it is often wavy in appearance, whereas the exochorion always has the slight curvature one would expect of a rigid shell layer. In the preparation of paraffin sections the two layers are sometimes, though not often, separated from one another.

At about 36 hours of incubation, which is about the time water absorption begins, the endochorion is broken up into many small fragments, which in surface view (Fig. 2) appear mainly rectangular in shape; in cross-section (Fig. 1, B–E), definite gaps in the endochorion can be clearly seen. The fragments still stain red with Mallory's. From Fig. 2 it may be seen that the fragments are organized into groups, all those in a single group having more or less the same 'long axes'. The orientation within a group is apparent from the size and contiguity of the spaces between the fragments as well as from their shape. If the egg is rinsed with chloroform for 30 seconds, the fragments appear in greater contrast with the spaces (probably a result of removing lipoid from the serosal cuticle), and the random orientation of the groups of fragments can be seen clearly. A group of fragments is about 10–20 μ across.

*Heymons (6) states that the chorion of *A. domesticus* is two-layered, and that the superficial one contains numerous irregular depressions. This is certainly not the case in the specimens used for the present study. Perhaps this is a racial difference.

†These terms are provisionally applied, until the origin of the layers is determined. Beament (1) defines chorion as "that part of the egg lying outside the oöcyte membranes, which is secreted by the follicle".

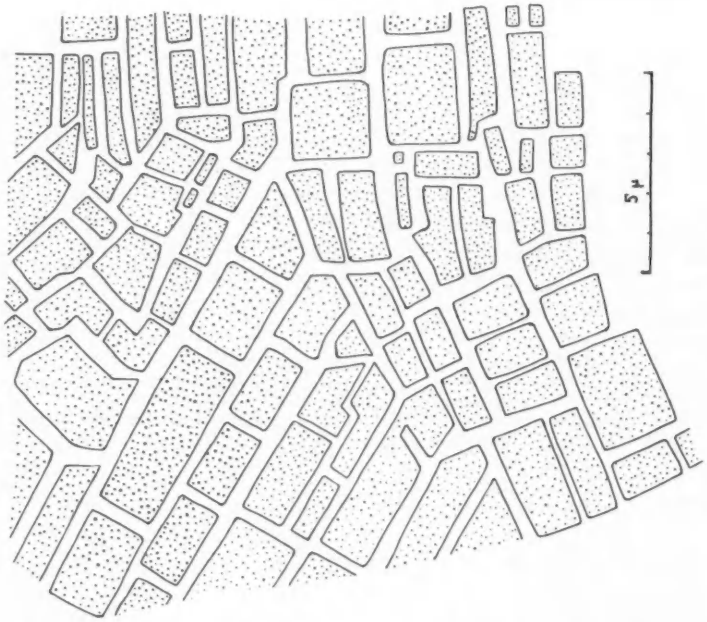

FIG. 2. Surface view of the fragmented endochorion showing the crystalloids.

As this is in the order of *cell* size, it seems quite possible that the material comprising each group is secreted by a single follicle cell, and hence that the endochorion is truly 'chorionic'.

The chorion probably functions as a mold for the serosal cuticle while the first layers of the serosal cuticle are being laid down, but shortly after water absorption has begun (48 hours) the chorion can be removed without affecting development. This may be done by immersing the eggs in sodium hypochlorite (Javex) for 3 minutes (with agitation): sections show that this treatment removes the chorion completely, and surface examination of the shells reveals the absence of the endochorionic fragments. The shell of eggs 0–36 hours old disintegrates rapidly, although not all eggs break up within 3 minutes, no doubt because the endochorion has not yet fragmented and is therefore more resistant.

Apart from support and protection for the egg, the chorion probably also provides the principal barrier to the passage of water and dissolved substances. Indeed, the visible change in the lipoid endochorion can leave little room for doubt that it is the breaking up of this layer which permits the egg to absorb water, and that it is this layer which functions as the barrier before water absorption begins.

This fragmentation of the endochorion could be explained in a number of ways, among them the following: (1) it is a mechanical breaking up, perhaps

due to the pressure of the swelling egg; (2) it is due to a chemical process which resembles crystallization; and (3) it is due to the dissolving away of interfragmental material (which could probably not be free lipoid, otherwise paraffin sections of 0- to 36-hour eggs would show the fragments). There is good evidence that the second explanation is the correct one.

Evidence for Natural Tanning of the Chorion

Eggs at any stage of development will darken when immersed in a saturated solution of tyrosine for 12 hours (10). This darkening is more intense during the first 36 hours than later. The darkening is confined to the chorion, as may be seen by immersing darkened eggs ($\geqslant$48 hours) in sodium hypochlorite: within 3 minutes the egg shells have become almost transparent. As this darkening is no doubt due to the formation of 'melanin', the presence of a tyrosinase* in the chorion is indicated.

Sections were made of 0-hour eggs which had been immersed in saturated tyrosine for only a few hours, before the entire chorion had blackened. Darkening was found to begin, and to be most intense, in the region of the endochorion. The tyrosinase is therefore probably located in this layer.

Natural tanning may or may not be accompanied by a browning of the material undergoing tanning. In the egg of *A. domesticus*, a slight but readily perceptible browning of the egg shell occurs at about the time water absorption begins, i.e., at 36 hours. This brownish color disappears on dechorionation of eggs 48 or more hours old, which reveals the whitish transparent serosal cuticle beneath.

The endochorion of 0-hour eggs cannot be broken up artificially by treatment of the shells with *p*-benzoquinone, at least in a way that is similar to the fragmentation occurring normally. The treated shells turn a light reddish-brown color, the color being apparently confined to the region of the endochorion; however, any browning of the treated shells to the extent that browning occurs in normal eggs (see above paragraph), that is, browning which is presumably due to quinone combined in tanning, would be masked by the color due to the uncombined quinone.

Effect of Phenylthiourea and Other Cu-*Enzyme Inhibitors*

Immersion of the eggs in a saturated solution of phenylthiourea for 12 hours prevents development of all 0-, 12-, and 24-hour eggs, and of most 36-hour eggs (at this time, some of the eggs have begun to take up water (10)), but does not affect the development of eggs 48 or more hours old.

That this effect is at least in part on the chorionic tyrosinase is shown by the fact that 12-hour eggs immersed in saturated phenylthiourea for 12 hours followed by immersion in saturated tyrosine show only a slight greying of the

*The term 'tyrosinase' is used in this paper to describe any enzyme(s) which will oxidize tyrosine, as judged by the formation of melanin, without implying that tyrosine is the natural substrate for the enzyme. Burris and Little (3), following Nelson and Dawson, prefer the term tyrosinase to polyphenol oxidase, catechol oxidase, phenolase, etc., 'on the basis of priority and because it does not emphasize the activity on polyphenols to the exclusion of the monophenols'.

shells as compared with the blackening of the control eggs. A similar experiment with 67-hour eggs, the development of which is not affected by phenylthiourea, gave similar results.

Twelve-hour eggs treated for 12 hours with phenylthiourea also do not show the fragmented endochorion when examined 48 hours after treatment. None of the eggs die after swelling normally, and any swelling that does occur seems to be post mortem.

It has not been possible to reverse the effect of 12-hour treatment with phenylthiourea by subsequent 12-hour treatment with 0.01 or 0.05 *M* copper chloride (0.1 *M* kills the eggs).

Other Cu-enzyme inhibitors were first tested in concentrations approximately equimolar with saturated phenylthiourea, which has a solubility of 0.26 g per 100 ml at 18° C (7). Two compounds (naphthylthiourea, salicylaldoxime) were found to be too insoluble to give equimolar solutions, and the saturated solution was used. The other compounds were tested at a variety of concentrations. The salicylaldoxime solution was prepared usually only 4 hours in advance of use, as it forms an oil in about 24 hours on standing at room temperature. Where mortality was complete among eggs treated with the inhibitors before water absorption took place, none of the eggs swelled normally.

1-Allyl-2-thiourea, in 0.1% concentration (equimolar is about 0.2%), has the same effect as phenylthiourea. Sixty-six-hour eggs are not affected by 24-hour immersion in 3.2% allylthiourea.

Thiourea kills 0-hour eggs after 24-hour treatment in 0.13% concentration. A 0.52% concentration has no effect on 67-hour eggs after 10-hour treatment.

Sodium diethyldithiocarbamate is ineffective in 0.29% solution (equimolar) on 0-hour eggs treated for 10 hours, but will kill 0-hour eggs treated for 24 hours with 0.58% solution. A 0.58% solution is ineffective on 67-hour eggs treated for 10 hours.

1-Naphthyl-2-thiourea is about 50% effective in killing 0-hour eggs immersed for 10 hours in the saturated solution.

Salicylaldoxime in saturated solution kills eggs of any age immersed for 24 hours. It will kill 12-hour eggs immersed for 10 hours. Eggs of 0, 12, and 24 hours are all swollen and white after treatment, whereas any effect of the other inhibitors is not visible to the naked eye.* All 67-hour eggs are killed after 10-hour treatment. Further effects of salicylaldoxime are discussed later.

Conclusion

The evidence suggests that the change in structure of the endochorion, brought about by phenolic tanning, increases the permeability of the shell and thereby permits water to be absorbed by the egg.

It is likely that the endochorion alone tans. The tyrosinase appears to be located in this layer, and, assuming that the precursors of the tanning agents do not enter the shell until just before tanning occurs, there could be no tanning quinones formed in the exochorion.

*Salicylaldoxime gives a particularly flocculent precipitate when mixed with aqueous copper chloride.

The breaking up of the endochorion resembles crystallization, inasmuch as the fragments formed have plane surfaces, and a similar shape (most are rectangular parallelopipeds). The fragments also stain in exactly the same way with Mallory's, i.e., a bright red, as the unfragmented endochorion. Although the breaking up of the endochorion is probably due to phenolic tanning, the resemblance of the process to crystallization is noteworthy, and for this reason the fragments have been termed 'crystalloids'. The basic pattern of the crystalloids probably exists in the unfragmented endochorion, and is only revealed by the subsequent tanning.

The Serosal Cuticle

The serosal cuticle is secreted, and water is absorbed, while the serosa surrounds the yolk and embryo (10, Fig. 1). During revolution the serosa is ruptured (6) so that secretion of the serosal cuticle ends with the completion of revolution. The serosal cuticle is thereafter steadily resorbed, and only a thin outer layer remains at the time of hatching of the egg (Fig. 1).

In surface view the serosal cuticle has a milky transparency, the milkiness being due to the presence of a highly refractive layer about 0.4 μ thick. This layer stains deeply with both Sudan stains. It is the only part of the serosal cuticle which is not resorbed by the embryo.

The inner layer of the serosal cuticle is apparently uniform in structure. As it stains a deep blue with Mallory's, this layer probably contains the chitin which is present in the serosal cuticle, as shown by Campbell's test (4). The thickness of the inner layer as determined from frozen sections (it shrinks greatly during paraffin embedding) is about 8–10 μ (minimum observed thickness).

The lipoid layer is present from 48 hours on and is presumably the first layer secreted by the serosa: eggs 48 or more hours old, when dechorionated with sodium hypochlorite, become quite hydrophobic. There is, then, a lipoid layer on the surface of the serosal cuticle while the egg is absorbing water. Microscopic examination of this layer in surface view and in section has not revealed any change in structure from 48 hours to hatching.

However, when eggs 72–96 hours old, after dechorionation, are rinsed with chloroform, for as few as 10 seconds, the shells almost immediately begin to blacken, in a more or less uniform manner, the blackening proceeding from the cuticle to the serosa (the outline of the serosal cell nuclei and the hexagonal cell boundaries become apparent) and then to the yolk. Darkening of the cuticle and serosa is uniform when the eggs are rinsed for 2–3 minutes. There seems to be a breakdown in permeability, as what appears to be yolk is frequently observed to exude from the surface, and many of the eggs collapse. The eggs appear also to become much more hydrophilic.

Eggs 120 or more hours old when treated with chloroform for 3 minutes show a similar blackening of the shell followed by a blackening of the yolk.

(In eggs close to hatching, the embryo presses against the shell, and the only free space remaining for the 'yolk' occurs between parts of the body and along intersegmental membranes. Blackening of the 'yolk' in these eggs results in the external form of the embyro being outlined in black.)

While the chloroform treatment does remove some of the lipoid, it does not remove all, as lipoid can still be demonstrated in the serosal cuticle of eggs 72 hours old (during water absorption) and 144 hours old (after water absorption). The blackening that follows chloroform treatment does not occur when dechorionated eggs are rinsed for 3 minutes with ethyl ether, benzene, xylene, or toluene: subsequent treatment with chloroform will blacken them.

These observations suggest that a tyrosinase is present in the serosal cuticle, and that it is located in the lipoid layer, as darkening will occur when dechorionated eggs are treated with chloroform just before hatching.

Dechorionated 48-hour eggs when treated with chloroform do not blacken: the phenolic sources of the melanin are therefore not present during the early part of the water absorption period.

Normal eggs treated with chloroform for 2 minutes for the most part survive (unlike dechorionated eggs, all of which die), but a few burst at about 120 hours, that is, at a time when water absorption should have ceased. In these eggs, the mechanism stopping water absorption does not operate, but permeability is not affected, as the eggs continue to swell and do not collapse.

That the darkening which follows chloroform treatment is enzymic is shown by the following experiment: If dechorionated eggs, 84 hours old, are rinsed with chloroform for 1 minute and then placed in 0.1 *M* KCN, the darkening, which has usually begun, proceeds no further, whereas eggs transferred to distilled water continue to blacken. The presence of a metallic enzyme is therefore indicated.

If eggs 48 or more hours old are dechorionated, and the contents (including the serosa) removed, the shells when incubated in saturated tyrosine blacken, but they will not blacken if they are first heated (3 minutes in boiling water), or in the presence of 0.1 *M* KCN. A tyrosinase is therefore present in the serosal cuticle, and as dechorionated shells of eggs just before hatching will blacken when treated in this way, the tyrosinase is located in the lipoid layer.

The serosal cuticle, at least for the most part, cannot be artificially tanned. Dechorionated shells from eggs 96 hours old (near the end of the water absorption period) and 120 hours old (after water absorption), after immersion for a few hours in *p*-benzoquinone, show a reddening which does not deepen after 48 hours of incubation at 35° C, but do not show the browning which is characteristic of tanning.

As chloroform removes some of the lipoid, the blackening of dechorionated eggs following chloroform treatment might be due simply to the admission of sufficient oxygen to permit the reaction to take place. If so, raising the oxygen tension of the environment could cause blackening. However, dechorionated eggs do not blacken when, for 20 minutes, oxygen is bubbled through distilled water containing the eggs.

Effect of Salicylaldoxime

Eggs of any age immersed in a saturated solution of salicylaldoxime for 20 hours do not survive the treatment. Whereas the effect on 0- to 36-hour eggs is no doubt at least in part due to the inhibition of the chorionic tyrosinase, the effect on eggs 48 or more hours old is, at least in part, on the tyrosinase of the serosal cuticle, as will be shown. (It will be recalled, from a preceding section, that salicylaldoxime is the only Cu-enzyme inhibitor tested which will visibly affect eggs 48 or more hours old, at least under comparable conditions of treatment and in concentrations which will not introduce serious osmotic effects.)

The effect of salicylaldoxime on eggs 60 or more hours old is very similar to the effect of the chloroform rinse on dechorionated eggs: first the serosal cuticle blackens, then the serosa, and finally the yolk. The blackening does not begin until about the 16th hour of immersion, and sections of eggs which have just begun to blacken show that the blackening is at first confined to the *inner* region of the inner serosal cuticle, adjacent to the serosa, although the serosa is not itself black. Subsequently the blackening spreads throughout the serosal cuticle (but not into the chorion), to the serosa, and finally to the yolk.* Eggs of 48 hours treated with salicylaldoxime do not blacken: this supports the above-mentioned conclusion that the substrate for the tyrosinase of the serosal cuticle is not present at this time.

Conclusion

The evidence presented above suggests the following explanation for the ending of water absorption and for the decrease in permeability of the egg to water after water absorption ceases: the lipoid layer of the serosal cuticle tans when revolution is completed, causing (1) a loss of water by the egg as its volume is reduced (9, 10), (2) a decrease in permeability of the shell as the lipoid becomes uniformly spread over the surface, and (3) an increase in the rigidity of the shell which creates a hydrostatic pressure sufficient to overcome the osmotic pressure of the yolk and embryonic fluid, and thus prevent bursting; and which also, in the event that the egg is exposed to an atmospheric humidity below saturation, creates a suction pressure tending to oppose water loss.

The function of the inner layer of the serosal cuticle is then primarily to provide an extensible support for the egg as it is swelling, although it may also contain enzymes which metabolize the precursors of the tanning agents (5). But, at any rate, its usefulness as a part of the shell is over when water absorption has ended, and it is resorbed. It is this layer which most probably contains the chitin. Apart from there being no observable change in the structure of this layer, except in thickness, the fact that it *is* resorbed shows, at least, that it does not undergo phenolic tanning.

*About 50% of the eggs of each age for which observations were recorded—72, 96, 120, and 144 hours—were split along the hatching line, although not greatly swollen. This splitting should probably not be interpreted as a bursting which results from the specific inhibition of the mechanism stopping water absorption, but rather as an effect peculiar to salicylaldoxime. It will be recalled that 0- to 36-hour eggs treated with salicylaldoxime swelled; swelling of eggs of this age cannot be due simply to an inhibition of the chorionic tyrosinase, as the other Cu-enzyme inhibitors do not have this effect.

The lipoid layer of the serosal cuticle is the first layer laid down by the serosa; it is formed at the time water absorption begins, and it contains lipoid and a tyrosinase from the time it is first laid down. As water is absorbed through the lipoid layer, it is not unreasonable to suppose that during the period of water absorption, the lipoid is discontinuous. If a change in this layer occurred, such that the lipoid came to form a continuous cover on the surface of the serosal cuticle, then water absorption would cease, or at least be greatly diminished. This change could conceivably take place through phenolic tanning of lipoprotein. The presence of a tyrosinase in the lipoid layer suggests that this might be the case.

The tyrosinase does not function when it is formed, according to this interpretation, because of the absence of substrates for it. These only appear 24 hours after water absorption begins. The nature of the phenolic substances causing blackening of the eggs after dechorionation and chloroform treatment has not been determined. However, blackening is so rapid that it seems likely they are polyphenols. Therefore, in addition to the tyrosinase of the lipoid layer, there is probably a tyrosinase in the yolk and perhaps also in the serosa. As isolated serosal cuticles (in distilled water) darken only very slowly, the tyrosinase in the serosal cuticle is probably confined to the lipoid layer.

Rinsing dechorionated eggs with chloroform removes lipoid, and this leads to blackening perhaps simply by the admission of oxygen, in the way that exposure to air causes blackening of raw cut potatoes (3). Or a lipoid barrier separating enzyme and substrate could be removed by the treatment.

The reason for the blackening of salicylaldoxime-treated eggs is not readily apparent, but may be due to the dispersion of the lipoid, as a result of the reaction between the copper ions and salicylaldoxime, admitting sufficient oxygen to cause the possibly non-enzymic melanin formation. However, raising the oxygen tension is not alone sufficient to cause blackening of the eggs, although it is possible the treatment was not continued long enough.

The immediate cause of the tanning of the lipoid serosal cuticle may be the stretching of this layer, as a result of the swelling of the egg, to a point where sufficient oxygen is available for the tyrosinase to form the tanning quinones. Or it may be due to the rupture of the serosa which permits free entry of the precursors of the tanning agents from the yolk into the cuticle, where they now reach a concentration which will permit tanning to occur.

In support of the latter view, two observations may be of significance. The first is that the majority of eggs after water absorption has been completed have a larger anterior than posterior end. This is not found in younger eggs, and as it is at the posterior end that the serosa first disappears (6), hardening of the cuticle at this end before the other could account for the change in shape. The second observation is the fate of two eggs which failed to revolve, and which were kept in distilled water. Both developed eye spots, but the yolk did not break down. Both swelled more than normal eggs, especially at the anterior end, and both finally burst, at the anterior end.

Discussion

It is possible that the tanning of lipoprotein membranes may be of widespread importance in insect eggs that absorb water after being laid, in breaking down the previous relative impermeability of the egg to water, so that water can enter; or in erecting a barrier (impermeable, hydrostatic) to the passage of water, which will stop water absorption; or in both. For example, the failure of diapause eggs of *Melanoplus differentialis* to regulate their water content (they burst) if placed in contact with water after xylol treatment (Slifer, cited by Lees (8)) may be due to the failure of such a mechanism as probably stops water absorption in *A. domesticus*. On the other hand, Laughlin (cited by Beament (2)) has found no lipoid in the egg shell of *Phyllopertha horticola*, the eggs of which absorb water. However, this does not exclude the possibility that tanning mechanisms are operating to control water absorption.

The theoretical possibility of using Cu-enzyme inhibitors as ovicides for the control of *A. domesticus* is strongly indicated by these observations. Phenylthiourea is not repellent to *A. domesticus*, and females will lay eggs normally in sand moistened with a saturated solution of the compound. In one test, only seven nymphs were obtained from several hundred eggs laid in such treated sand (McFarlane, unpublished observation). No doubt other crickets, and possibly many other insects, would be similarly susceptible. The widespread distribution and probable importance of tyrosinase in plants (3) does not, at the moment, preclude the possibility of the use of these inhibitors against plant pests.

Acknowledgments

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References

1. BEAMENT, J. W. L. The formation and structure of the chorion of the egg in an hemipteran, *Rhodnius prolixus*. Quart. J. Microscop. Sci. **87**, 393–439 (1946).
2. BEAMENT, J. W. L. Water transport in insects. Symposium Soc. Exptl. Biol. **8**, 94–117 (1954).
3. BURRIS, R. H. and LITTLE, H. N. Oxidases, peroxidase, and catalase. *In* Respiratory enzymes. H. A. Lardy, *Editor*. Burgess Publishing Co., Minneapolis 1949.
4. CAMPBELL, F. L. The detection and estimation of insect chitin; and the irrelation of "chitinization" to hardiness and pigmentation of the cuticula of the American cockroach, *Periplaneta americana* L. Ann. Entomol. Soc. Am. **22**, 401–426 (1929).
5. DENNELL, R. The hardening of insect cuticles. Biol. Revs. **33**, 178–196 (1958).
6. HEYMONS, R. Die Embryonalentwickelung von Dermapteren und Orthopteren. Gustav Fisher, Jena. 1895.
7. HODGMAN, C. D. (*Editor*). Handbook of chemistry and physics. 33rd ed. Chemical Rubber Publishing Co., Cleveland. 1951.
8. LEES, A. D. The physiology of diapause in arthropods. Cambridge University Press. 1955.
9. McFARLANE, J. E., GHOURI, A. S.K., and KENNARD, C. P. Water absorption by the eggs of crickets. Can. J. Zool. **37**, 391–399 (1959).
10. McFARLANE, J. E. and KENNARD, C. P. Further observations on water absorption by the eggs of *Acheta domesticus* (L.). Can. J. Zool. **38**, 77–85 (1960).
11. PANTIN, C. F. A. Notes on microscopical technique for zoologists. Cambridge University Press. 1948.

THE METAZOAN PARASITES OF THE HETEROSOMATA OF THE GULF OF ST. LAWRENCE

V. MONOGENEA[1, 2]

KEITH RONALD[3]

Abstract

Udonella caligorum, *Entobdella hippoglossi*, and *E. curvunca* were identified in a study of 43 Atlantic halibut, *Hippoglossus hippoglossus*. The incidence, distribution, and host specificity of the halibut's monogenetic parasites are discussed.

Introduction

Although 100 Atlantic halibut (*Hippoglossus hippoglossus* (Linné, 1758)) were examined for endoparasites only the 43 examined externally are discussed here.

In this, as in previous papers (33, 34), mention should be made of the loss of parasites that occurs in examining fish taken by commercial methods. The possible transport of an ectoparasite to an abnormal host, due either to fish-handling techniques or to the parasite leaving the host as the latter is removed from the water, must be considered. For this reason, only 43 specimens of halibut are discussed. While the incidence figures are comparable to those in other reports absolute accuracy cannot be claimed.

Methods

After careful removal from the host, trematodes were placed in an isotonic solution (2 parts sea water to 1 part distilled water) and left overnight to relax. After the trematodes were fixed in Gilson's fluid (1898) for not less than 1 day, they were washed for from 1 to 2 days. The specimens were stained with Mayer's carmalum diluted in the ratio of 1:100 with 5% potassium alum. This procedure necessitated holding the specimens in the staining solution for as long as 1 month. Thymol was added to inhibit fungus growth. After they were stained the trematodes were rinsed with 5% alum. They were bleached with dilute potassium permanganate, washed, then placed in 4% oxalic acid for a few seconds, and returned to distilled water. After dehydration, the specimens were cleared in 1,4-dioxane and mounted in a synthetic naphthalene polymer.

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Suborder MONOPISTHOCOTYLEA
Superfamily CAPSALOIDEA
Family Udonellidae

Udonella caligorum Johnston, 1835

Host: *Lepeophtheirus hippoglossi* (Krøyer, 1837).
Locality: South West Point, Anticosti Island, P.Q.

Udonella caligorum has been reported (1, 4, 17) as a parasite of *Caligus* sp. on *H. hippoglossus*, and on the same genus of copepod parasitic on *Platichthys flesus* (27). These records are from the eastern Atlantic and Mediterranean. There is only one record (15) of *U. caligorum* on *H. hippoglossus* from North American waters.

The present record is of interest because the number of parasites found on the copepod far exceeds that recorded elsewhere. It was found on 5 of 32 female and 11 of 14 male *L. hippoglossi* present on a single halibut. The male copepod was parasitized by both young and mature trematodes, the young always on the ventral surface of the carapace and the few mature forms attached to the lateral and dorsal surfaces. On the female copepod, *U. Caligorum* was always attached to the ovisacs, usually grouped laterally or on the distal end, giving the ovisac a typical "lion's tail" appearance.

The male *L. hippoglossi* carried 15 to 74 trematodes usually clustered around the second and third pairs of biramose legs. The female carried 8 to 26 trematodes on the ovisacs.

Udonella caligorum has been classified as a commensal (1). After removing the trematodes in the present study I noticed that the ovisac was damaged and that there were many small indentations along its length, suggesting that the copepods were being used as a source of food by the trematode.

The adult trematodes ranged from 2.4 to 3.5 mm in length and from 0.38 to 0.56 mm in breadth. The immature forms varied in size, the shortest being 0.35 mm, and the longest 2.5 mm.

Family Capsalidae

Entobdella hippoglossi (Müller, 1776)

Host: Hippoglossus hippoglossus.
Location: Skin.
Locality: South West Point, Anticosti Island; Brion Island, Magdalen Islands, P.Q.

Entobdella hippoglossi is one of the most common and widely distributed ectoparasites of the halibut. It has been recorded as a parasite of Atlantic halibut 22 times, less frequently from the smaller Pacific halibut, *Hippoglossus stenolepis* Schmidt, 1904 (18, 23, 25, 42).

From one to five trematodes were found on 40% of the halibut examined from the Gulf of St. Lawrence.

The measurements of my specimens differ from those of Price (30). Dawes (7) noted a similar difference and stated in a footnote: "Price may be in error

regarding the breadth of the body and in the size of the opisthaptor ...." My specimens measure from 12.0 to 17.5 mm in length by 6.0 to 7.0 mm in breadth. Body shape irregularly elliptical with indentations between cephalic lobe and body. Opisthaptor comparatively large, 3.5 to 4.7 mm in diameter. Three pairs large hooks (anchors) with 14 marginal hooklets (haptorial hooks). The three pairs of hooks resemble closely those described for this species (6, 30).

Of the specimens examined 23% were gravid, the tetrahedral eggs bearing long filaments.

Entobdella curvunca Ronald, 1957

Host: Hippoglossus hippoglossus.

Location: Skin.

Locality: Latabatière, north shore of the Gulf of St. Lawrence; Anticosti Island; Brion Island, Magdalen Islands, P.Q.

The description and incidence of this parasite have been recorded elsewhere (33).

Discussion

The halibut is possibly parasitized by five genera of monogenetic trematodes in each of which there is no sharply defined host specificity, or even supraspecificity (13).

Diclidophora palmata (Leuckart, 1830) has been recorded (31) from the Atlantic halibut and from the ling, *Gadus molva* (3, 20, 21, 24, 31, 32, 36, 37).

If Rathke's (31) identification of *D. palmata* is correct, then this trematode occurs on two host species with common habitats. In view of the single host records of other workers however, Rathke's identification would bear substantiation. It is doubtful if *D. palmata* is a parasite of the Heterosomata.

It is doubtful if *Entobdella hippoglossi* can be called species-specific because in addition to having been recorded many times on *H. hippoglossus*, there is also a dubious reference to it having been found on a gadoid from North American waters (Canavan in Sproston (38)). Although Sproston includes this record in her text (p. 325) she does not include it in her bibliography and I have been unable to trace the reference.

Similarly, *E. squamata* is referred to only in Sproston's (38) host list and nowhere else in her paper. If, as may be, this is a lapsus and her reference to *E. squamula* Heath, 1902 (= *E. squamata*) on *H. hippoglossus* is correct, it undoubtedly refers to Guberlet's (12) record, and this species too shows little if any host specificity. The other hosts of *E. squamula* range from a flatfish, *Paralichthys californicus*, to *Sebastodes* sp. (14) to an unidentified species from the Gulf of Mexico (30).

It would appear as if *E. curvunca* is the only really host-specific species in the Heterosomata – monogenetic trematode complex, but this may merely be due to our lack of knowledge of the parasite fauna of allied hosts in the northwest Atlantic.

Megalocotyle rhombi (van Beneden and Hesse, 1863) is usually regarded as a parasite of the European turbot, *Scophthalmus maximus* (3, 26, 29), but the halibut too has been implicated (4). As both are benthic hosts this compatibility is understandable.

Tristoma uncinatum Monticelli, 1889, another parasite of the halibut (4), was recorded originally from *Pleuronectes* sp. (27). This trematode might be host-specific but because of the taxonomic confusion in the host records no conclusion can be drawn at this time.

The hyperparasite, *Udonella caligorum*, falls into a different category because it is not genus-specific and has been found attached to copepods of the genus *Caligus* living on *H. hippoglossus* (1, 2, 4, 16, 17, 18), and as recorded here and elsewhere (34) as a parasite of *Lepeophtheirus hippoglossi* and also attached directly to a fish (38).

Infected copepods have also been reported from the European flounder, *Platichthys flesus* (27), gadoids (1, 2, 9, 10, 19, 22, 23, 28, 35, 37, 39, 40), and other groups (5, 8, 11, 41).

The status of the specificity of the monogenetic trematodes parasitizing Atlantic halibut is confused insofar as the literature is concerned and there is little evidence that the monogenetic trematodes follow discreet lines of parasitism in respect to *Hippoglossus hippoglossus*.

References

1. VAN BENEDEN, P. J. Mémoire sur les vers intestinaux. Suppl. Compt. rend. II (1858).
2. VAN BENEDEN, P. J. Les poissions des côtes Belgique, leurs parasites et les commensaux. Mém. acad. roy. soc. Belg. **38** (1871).
3. VAN BENEDEN, P. J. and HESSE, C. E. Recherches sur les bdellodes ou hirudinees et les trématodes marins. Bruxelles (1863).
4. BRAUN, M. G. C. C. Einige. Bemerkungen über die Körperbedectung ektoparasitischer Trematoden. Centr. Bakteriol. **7**, 594–598 (1890).
5. DALYELL, J. G. The powers of the creator displayed in creation; or observations on life admist the various forms of the humbler tribes of animated nature with practical comments and illustrations. London, 1 and 2 (1853).
6. DAWES, B. The Trematoda, with special reference to British and other forms. Cambridge (1946).
7. DAWES, B. The Trematoda of British fishes. London (1947).
8. DELAMARE-DEBOUTTEVILLE, C. *Udonella caligorum* Johnston (1835), trematode monogénétique, phorétique du copépode *Caligus minimus* Otto. Vie et milieu. Bull. Lab. Arago, **1**, 362–363 (1950).
9. DIESING, K. M. Systema helminthum, **1** Vindobonae (1850).
10. FREY, H. and LEUCKART, R. Beitrage zur Kenntniss wirbellosen Thiere mit besonderer Berucksichtigung der Fauna des Norddeutschen Meeres. Braunschweig, **4** (1847).
11. GIARD, A. Sur un copépode (*Cancerilla tubulata* Dalyell) parasite de l'*Amphiura squamata* Delle Chiaje. Compt. rend. 1189–1192 (1887).
12. GUBERLET, J. E. Trematodos ectoparasitos de los peces de la costas del Pacifico. Anales inst. biol. (Univ. nac. Méx.) **7**, 457–467 (1937).
13. HARGIS, W. J. Host-specificity of monogenetic trematodes. Exptl. Parasitol. **6**, 610–625 (1957).
14. HEATH, H. The anatomy of *Epibdella squamula*, sp. nov. Proc. Calif. Acad. Sci. Zool. 3, s., **3**, 109–136 (1902).
15. HOYLE, W. E. Trematoda. *In* Encyclopaedia Britannica. 9th ed. 1888. pp. 535–540.
16. JOHNSTON, G. Illustrations in British zoology. Mag. Nat. Hist. **8**, 494–498 (1835).
17. JOHNSTON, G. A catalogue of the British non-parasitical worms in the collection of the British Museum London. 1865.
18. JOHNSTON, T. H. Remarks on the synonymy of certain tristomatid trematode genera. Trans. Proc. Roy. Soc. S. Australia, **53**, 71–78 (1959).
19. KRØYER, H. Om Snyltekrebsene, isoer med Hensyntil den danske. Naturhist. Tidsskr. Kjøbenhavn, **1** (1837).

20. LEBOUR, M. V. Fish trematodes of the Northumberland coast. Rept. Sci. Invest. Northumberland Sea Fish Comm. 1907, 23–67 (1908).
21. LEUCKART, F. S. Trematoden. Isis, **6**, 612 (1830).
22. LINTON, E. Notes on trematode parasites of fishes. Proc. U.S. Natl. Museum, **20**, 507–548 (1898).
23. LINTON, E. Trematodes from fishes mainly from the Woods Hole region, Massachusetts. Proc. U.S. Natl. Museum, **88** (1940).
24. LITTLE, P. A. The trematode parasites of Irish marine fishes. Parasitology, **21**, 22–30 (1929).
25. MARGOLIS, L. Studies on parasites and diseases of marine and anadromous fish from the Canadian Pacific coast. Thesis, McGill University, Montreal, Que. 1952.
26. MASSA, D. Contributo allo studio del genere Trochopos. Monit. zool. ital. **14**, 252–255 (1903).
27. MONTICELLI, F. S. Elenco degli elminti studiati a Wimereux nella primavera del 1889. Bull. sci. France et Belg. **22**, 417–444 (1890).
28. MONTICELLI, F. S. Il genere Lintonia Monticelli. Arch. zool. Napoli, **2**, 117–124 (1904).
29. PARONA, C. and MONTICELLI, F. S. Sui generi *Placunella* e Trochopus. Monit. zool. ital. **13**, Suppl., Dic., 46–48 (1902).
30. PRICE, E. W. North American monogenetic trematodes III. The family Capsalidae (Capsaloidea). J. Wash. Acad. Sci. **29**, 63–92 (1939).
31. RATHKE, H. Beiträge zur Fauna Norwegens. Nova Acta Acad. Nat. Curios. **20**, 1–264c (1843).
32. REES, F. G. and LLEWELLYN, J. A record of the trematode and cestode parasites of fishes from the Porcupine bank, Irish Atlantic slope and Irish Sea. Parasitology, **33**, 390–396 (1941).
33. RONALD, K. The metazoan parasites of the Heterosomata of the Gulf of St. Lawrence. II. *Entobdella curvunca* sp. nov. (Trematoda:Capsalidae). Can. J. Zool. **35**, 747–750 (1957).
34. RONALD, K. The metazoan parasites of the Heterosomata of the Gulf of St. Lawrence. III. *Copepoda parasitica*. Can. J. Zool. **36**, 1–6 (1958).
35. SAINT-REMY, G. Synopsis des Trématodes monogénèses. Rev. Biol. Nord France Lille, **6** (1892).
36. SCOTT, T. *Dactylocotyle palmatum* (Leuchart (sic)) in the Moray Firth. Ann. Scottish Nat. Hist **22**, 127 (1897).
37. SCOTT, T. Notes on some parasites of fishes. 19th Ann. Rept. Fisheries Board Scotland (1900), **3**, 120–153 (1901).
38. SPROSTON, N. B. A synopsis of the monogenetic trematodes. Trans. Zool. Soc. London, **25**, 185–600 (1946).
39. STAFFORD, J. Trematodes from Canadian fishes. Zool. Anz. **27**, 481–495 (1904).
40. STAFFORD, J. Preliminary report on the trematodes of Canadian marine fishes. Contrib. Can. Biol. (1902–05), 91–94 (1907).
41. VOGT, C. Recherches côtières. Premier mémoire-De la famille des Philichthydes et en particulier du Lepospaile des Labres (*Leposphilus labrei* Hesse). Second mémoire sur quelques Copépodes parasites à mâles pygmées habitant les poissons. Mém. inst. nat. genevois, **13** (1877).
42. WINTER, H. A. *Capsala caballeroi* sp. n., parásite de *Saida orientalis*, con un catalogo de los tremátodos monogéneos de los peces del océano Pacifico de las Américas. Rev. brasil. biol. **15**, 9–32 (1954).

THYROIDAL CONTROL OF RADIOPHOSPHORUS METABOLISM IN YEARLING SALMON, SALMO SALAR L.[1]

P. N. SRIVASTAVA[2]

Abstract

In one experiment yearling *Salmo salar* L. treated with 0.3% thiourea for a fortnight absorbed 50.3% of P^{32} from the water whereas in control they could utilize only 40.1%. The uptake in various tissues (bone, liver, kidney, and muscles) was always more in treated fishes than in control. In a second experiment the utilization of radiophosphorus was minimum (39%) in control, more (52%) in thiourea-injected fishes, and maximum (63%) in thyroxine-injected ones. The turnover time of P^{32} was slower in control than the treated fishes. It is suggested that in the thiourea-treated fishes stimulation of the thyroid is indirect (by thyrotropin secreted by the pituitary gland) whereas in the thyroxine-injected fishes the stimulation is direct and so the P^{32} utilization is maximum. Thus the thyroid stimulates mineral metabolism.

Introduction

The role played by the thyroid gland in teleost metabolism has been investigated by a large number of workers. In cold-blooded vertebrates, it appears to be concerned more with growth and differentiation than with general metabolism (12). However, there is a great deal of controversy on practically every aspect of thyroid physiology. Since detailed and excellent reviews of literature already exist (8, 15) it is not intended here to review the many ideas on the functions of thyroid in teleosts.

Root and Etkin (18), Etkin *et al.* (3) were unable to find any effect of thyroid extract or thyroxine on oxygen consumption of goldfish, and Hasler and Meyer (6) also obtained negative results. Müller (14) obtained a highly significant rise in the oxygen consumption of goldfish after a single injection of thyroxine. Smith and Everett (19) have shown that thyroxine or desiccated thyroid powder had no effect on growth rate, oxygen consumption, or the time of sexual maturity in males of newly born guppies. By contrast Smith and Matthews (20) observed an increased rate of oxygen consumption in white grunts, *Bathystoma*, after treatment with parrot fish, *Sparisma*, thyroid extract; and Smith *et al.* (21) state that in *Lebistes reticulatus*, treated with thiourea, the growth rate is markedly reduced and sexual differentiation is delayed in males.

La Roche and Leblond (11) administered various thyroid preparations, including thyroxine, to Atlantic salmon and observed a pallor and thickening of the integument which was slight in fry, moderate in parr, and intense in smolt. Since similar changes occur during the transformation of parr into smolt under natural conditions, the findings were consistent with the idea that secretion of the thyroid gland played a role in this transformation.

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In higher vertebrates, parathyroid through the action of its hormone, parathormone, is essential for the maintenance of the normal calcium and phosphorus content and distribution within the body (23). It has been suggested (17) that the ultimobranchial body in the teleost is the homologue of the parathyroid gland but as yet nothing positive is established regarding this hypothesis. In fishes, there is scarcely any information concerning the role of endocrines in the regulation of mineral metabolism. Fontaine and Callamand (unpublished quoted in (4)) state that after hypophysectomy in young eels (40–70 g) the calcium dropped by 16% six days after the operation and fell to 25% in 3 weeks. In larger eels (300–700 g) blood calcium dropped 15% during the first 2–6 days and fell to 28% in 10 days. Fish have, so far as known, no parathyroid and the present experiments were carried on to see whether, besides other functions, thyroid gland has any influence over their mineral metabolism.

Material and Methods

Yearling salmon, *Salmo salar* L., were secured from the rearing ponds at Grand Lake, Nova Scotia, through the courtesy of the Fish Cultural Development Branch of Canada. They were kept in the laboratory in well-aerated aquaria at $10 \pm 1°$ C for 2 days before starting any experiments. In fishes, the thyroid gland is divided into innumerable follicles in the region of the ventral aorta; hence surgical thyroidectomy is impossible. The alternative chosen was to destroy the gland by the antithyroid drug, thiourea. The fishes were divided into two groups, of which one was maintained in 0.3% solution of thiourea, referred to below as treated. The other was maintained as a control. The fishes were kept in thiourea solution for 2 weeks, the solution being changed every 3 days.

After 2 weeks the fish were transferred to fresh aquaria to which sufficient P^{32} had been added to give an activity of 2500 counts per minute per milliliter. Aquaria for treated fish also contained 0.3% solution of thiourea. It is known that when P^{32} is added to water in inorganic form, a considerable portion is converted to organic state and a condition of equilibrium is established in a day (5, 7). To permit equilibration the aquaria were held for 24 hours after addition of P^{32} before transferring any fish to them. At intervals of 2, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours, four fishes were taken from each set and the tissues (liver, kidney, muscles, and bone) were variously treated (22) and prepared for counting. The counting was done on a Geiger–Muller scaler using Geiger tube D34 (Nuclear-Chicago Corp.). The readings were corrected for decay and checked at intervals against a uranium standard. Radiophosphorus was received from Atomic Energy of Canada Limited in form of carrier-free H_3PO_4 in HCl. In all about 320 fishes had been used and the experiments were carried on twice. The results showed the same consistency.

Results

Figure 1 shows the uptake of radiophosphorus in both sets of experiments, bone taking up the maximum activity followed by liver, kidney, and muscles.

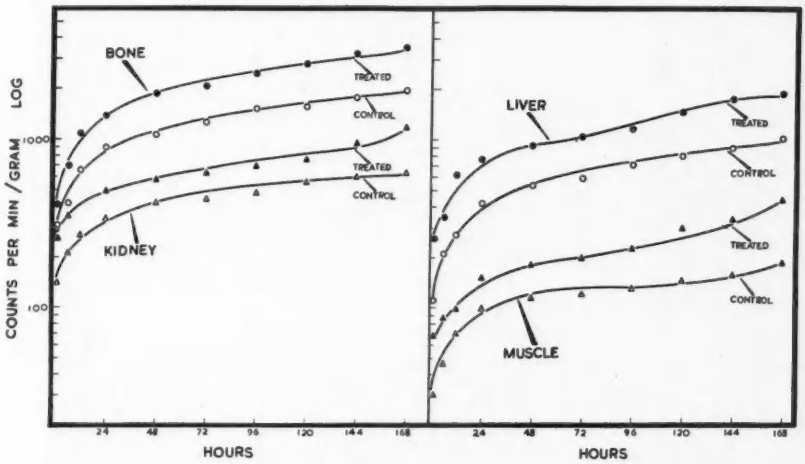

FIG. 1. Uptake of radiophosphorus in bone, kidney, liver, and muscle in control and fishes treated with 0.3% thiourea for 2 weeks. All the points represent the average of four estimations.

The uptake was always more in the treated set than in the control. The turnover time which may be defined as the time required to renew completely the amount of substance present in the tissue was found out by plotting the log activity of the tissues against time, drawing the best slope of the line, and then calculating it by the following formula:

$$t_t = (\log_e \times t)/(\log A_0 - \log A)$$

where t_t is the turnover time, log A_0 the log activity at time t, and log A the log activity at 0 time. The activity reached at 168 hours and the turnover time for P^{32} in both the sets are given in Table I.

TABLE I

P^{32} activity at 168 hours and turnover time for various tissues

Tissues	Activity at 168 hours, c.p.m./g		Turnover time, hours	
	Treated	Control	Treated	Control
Bone	3660	2045	21.3	12.2
Liver	1910	1005	23.8	15.7
Kidney	1195	665	27.3	19.1
Muscles	445	195	31.9	24.5

Decline of P^{32} in the aquaria was followed in both sets and their readings confirm the higher uptake in the treated set (Fig. 2). In control 40.1% of P^{32} was absorbed whereas in treated 50.3% was taken up.

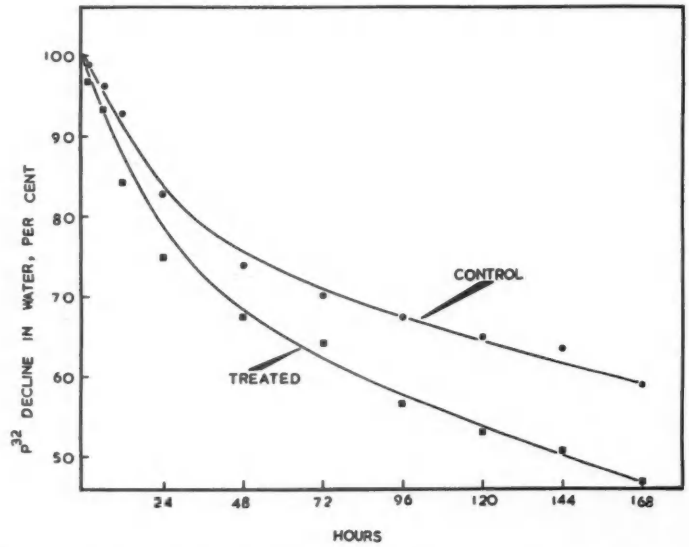

FIG. 2. Decline of radiophosphorus in the aquaria (Fig. 1). The points represent the average of four readings.

Another set of experiments was arranged to throw further light on the problem. The fishes were divided into three groups. One of them was injected with 0.2 ml of 0.1% solution of thiourea, the other with 0.2 ml of 0.1% solution of *l*-thyroxine (Nutritional Biochemical Corp.), and the third group with 0.2 ml physiological saline to serve as control. These injections were repeated every 3 days. In making up the solutions used for injection, enough sea water was added to the salt dissolved in water to bring the mixture to isotonicity with salmon coelomic fluid. Thus it was presumed that injections did not interfere with the maintenance of osmotic balance in the fish. After three injections of each, the fishes were transferred to solutions of P^{32} which had been prepared a day earlier for proper equilibration of phosphorus. The P^{32} activity in water was 2500 counts per minute per milliliter. In these experiments the uptake of radiophosphorus in bone and liver was studied after intervals of 2, 6, 12, 24, 48, 96, and 144 hours. The maximum activity reached and the turnover time for phosphorus are given in Table II and Fig. 3.

TABLE II

P^{32} activity at 144 hours and turnover time in bone and liver

	Activity at 144 hours, c.p.m./g			Turnover time, hours		
Tissues	Control	Thiourea	Thyroxine	Control	Thiourea	Thyroxine
Bone	1220	3335	6150	17.2	14.8	11.5
Liver	835	1420	2445	19.9	16.3	12.0

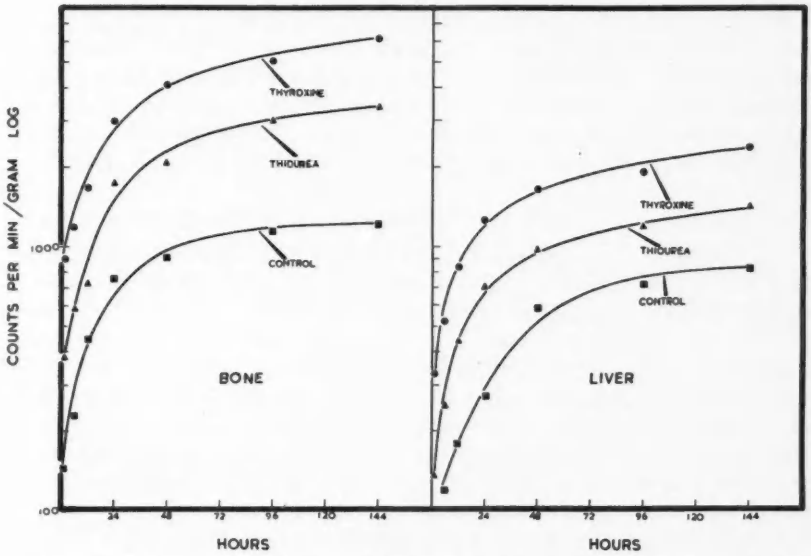

FIG. 3. Uptake of radiophosphorus in bone and liver in control, thiourea- and thyroxine-injected fishes. All the points represent the average of four readings.

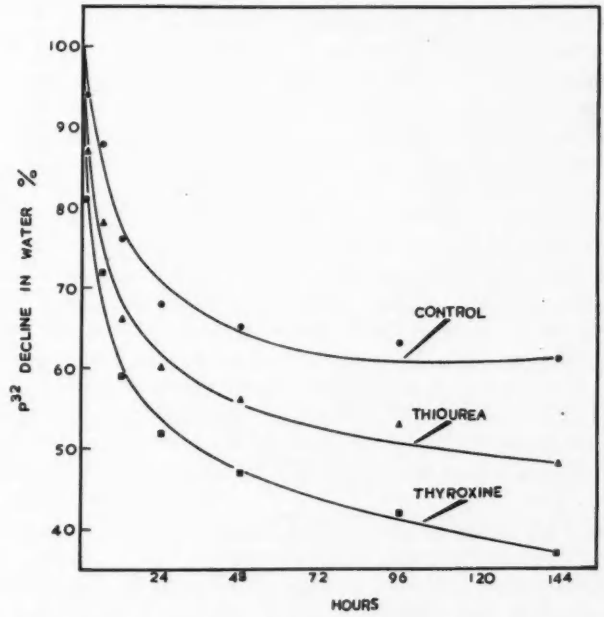

FIG. 4. Decline of radiophosphorus in aquaria (Fig. 3). The points are the average of four readings.

As compared with controls, the thiourea treatment produced the same result as before (Fig. 1). Loss of P^{32} from water was studied in these experiments as well. In the control the fishes took up 39%, fishes injected with thiourea absorbed 52%, and thyroxine-injected fishes utilized 63% of P^{32}. These results (Fig. 4) are in appropriate order to confirm the direct measurements on uptake by bone and liver shown in Fig. 3.

Discussion

The fishes in the foregoing experiments were treated with the thought that the thyroid gland would be destroyed and consequently the mineral metabolism would be affected in such a way that P^{32} uptake would be lowered. But the experiments gave results to the contrary, the real cause of which was revealed by the histological study of the thyroid. It showed that instead of being destroyed they were active. The follicles were lined with a single layer of low epithelial cells and were full of colloid as in any other normal follicle. Besides this, the hyperplastic nature of the gland because of the thiourea treatment was also apparent. There was an increase in the number of follicles throughout the length of the gland.

Astwood (1) stated that the administration of antithyroid drugs either abolishes or retards the ability of the thyroid gland to synthesize thyroxine. The physiological reaction does not end there. As a consequence of the cessation of thyroxine synthesis, the level of circulating hormone drops and the pituitary gland immediately responds by secreting additional amounts of thyrotropin which stimulates the thyroid so that hypertrophy of the thyroid results. This is what has exactly happened in the above experiments. Twenty-three species, both fresh-water and marine, have been investigated by various authors and the histological evidence of thyroid stimulation has been observed. The magnitude of response, however, varies with species and conditions of experiments (15). This shows that the pituitary can stimulate the thyroid by secreting additional amounts of thyrotropin although for what length of time is not exactly known. So if the thyroid gland has to be destroyed by antithyroid drugs, its treatment will have to be continued for a considerable length of time, until the pituitary body is completely exhausted of its thyrotropin. Only then the drugs will really have any effect on the thyroid. Moreover, Chapman (2), Morton *et al.* (13), Purves and Griesbach (16), Hum *et al.* (9) have shown that even the total removal or destruction of the thyroid gland does not wholly abolish the synthesis of thyroid hormone. It appears that small amounts of thyroxine can be synthesized even in the total absence of thyroid tissue, but the site of this extrathyroidal function has not yet been identified.

The results of these experiments can be interpreted on the basis of the above-mentioned facts that when fishes are treated with thiourea, the thyroid is stimulated indirectly (by the thyrotropin secreted by the pituitary body) and so the uptake of radiophosphorus is more than the control. In fishes treated with thyroxine, the stimulation is direct and stronger and so the

uptake is maximum. Müller (14) had also obtained striking results with goldfish, *Carassius auratus*, after a single injection of thyroxine. This caused a significant rise in oxygen consumption. It was also shown by him that the effect of thyroxine can be duplicated by the administration of thyrotropin. The present author feels that the thyroid gland has positive influence over the mineral metabolism of fishes in that its extract speeds up the turnover of phosphorus.

La Roche and Leblond (10) have shown that complete thyroidectomy can be achieved by repeated doses of radioiodine. Further work to study the effect of hypophysectomy and destruction of thyroid by radioiodine on mineral metabolism is under investigation and the results will be published later.

Acknowledgments

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References

1. Astwood, E. B. Mechanism of action of anti-thyroid compounds. Brookhaven Symposium Biol. **7**, 61–70 (1955).
2. Chapman, A. Extrathyroidal iodine metabolism. Endocrinology, **29**, 686–694 (1941).
3. Etkin, W. N., Root, R. W., and Mofskin, B. P. The effect of thyroid feeding on oxygen consumption of goldfish. Physiol. Zoöl. **13**, 415–429 (1940).
4. Fontaine, M. The hormonal control of water and salt electrolyte metabolism in fish. Mem. Soc. Endocrinol. **5**, 69–81 (1956).
5. Harris, E. Radiophosphorus metabolism in zooplankton and microorganisms. Can. J. Zool. **35**, 769–782 (1957).
6. Hasler, A. D. and Meyer, R. K. Respiratory responses of normal and castrated goldfish to teleost and mammalian hormones. J. Exptl. Zool. **91**, 391–404 (1942).
7. Hayes, F. R. and Phillips, J. E. Lake water and sediment. IV. Radiophosphorus equilibrium with mud, plants, and bacteria under oxidized and reduced conditions. Limnol. and Oceanog. **3**, 459–475 (1958).
8. Hoar, W. S. Endocrine organs. *In* the Physiology of fishes. Vol. 1. Academic Press, New York. 1957.
9. Hum, R. F., Goldberg, R. C., and Chaikoff, I. L. Effect of excess iodine upon anterior pituitary cytology of the completely thyroidectomized rat and its bearing on the question of extrathyroidal thyroxine synthesis. Endocrinology, **49**, 21–24 (1951).
10. La Roche, G. and Leblond, C. P. Destruction of thyroid gland of Atlantic salmon, *Salmo salar* L., by means of radioiodine. Proc. Soc. Exptl. Biol. Med. **87**, 273–276 (1954).
11. La Roche, G. and Leblond, C. P. Effect of thyroid preparations and iodide on Salmonidae. Endocrinology, **51**, 524–545 (1952).
12. Lynn, W. G. and Wachowski, H. E. The thyroidal gland and its function in cold-blooded vertebrates. Quart. Rev. Biol. **26**, 123–168 (1951).
13. Morton, M. E., Chaikoff, I., Reinhardt, W. O., and Anderson, E. Radioactive iodine as an indicator of the metabolism of iodine. VI. The formation of thyroxine and diiodothyroxine by the completely thyroidectomized animal. J. Biol. Chem. **147**, 757–769 (1943).
14. Müller, J. Über die Wirkung von Thyroxin und Thyreotropem Hormon auf den Stoffwechsel und die Farbung der Goldfisches. Z. vergleich. Physiol. **35**, 1–12 (1953).
15. Pickford, G. E. and Atz, J. W. The physiology of the pituitary gland of fishes. New York Zoological Society, New York. 1957.
16. Purves, H. D. and Griesbach, W. E. Observations on the acidophil cell changes in the pituitary of thyroxine deficiency states. I. Acidophil degranulation in relation to goitrogenic agents and extrathyroidal thyroxine synthesis. Brit. J. Exptl. Pathol. **27**, 170–179 (1946).

17. RASQUIN, P. and ROSENBLOOM, L. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull. Am. Museum Nat. Hist. **104**, 359–426 (1954).
18. ROOT, R. W. and ETKIN, W. Effect of thyroxine on the oxygen consumption of toadfish. Proc. Soc. Exptl. Biol. Med. N.Y. **37**, 174–175 (1937).
19. SMITH, D. C. and EVERETT, G. M. The effect of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish, *Lebistes reticulatus*. J. Exptl. Zool. **94**, 229–240 (1943).
20. SMITH, D. C. and MATTHEWS, S. A. Parrot fish thyroid extract and its effect upon oxygen consumption in the fish, *Bathystoma*. Am. J. Physiol. **153**, 215–221 (1948).
21. SMITH, D. C., SLADEK, S. A., and KELLNER, A. W. The effect of mammalian thyroid extract on the growth rate and sexual differentiation in the fish, *Lebistes reticulatus*, treated with thiourea. Physiol. Zoöl. **26**, 117–124 (1953).
22. SRIVASTAVA, P. N. Effect of bacteria on radiophosphorus metabolism in yearling salmon, *Salmo salar* L. In press.
23. THOMSEN, D. L. and COLLIP, J. B. Action of parathyroid hormone, review. Physiol. Revs. **12**, 309–383 (1932).

THE ORIGIN OF THE PROTUBERANCE ON THE INNER SURFACE OF THE EGG CAPSULE OF CYSTIDICOLA CRISTIVOMERI[1]

ROY C. ANDERSON

Abstract

The origin of the small elevation on the inner surface at one end of the egg capsule of *Cystidicola cristivomeri* has been investigated and it is concluded that it represents the first polar body.

Introduction

A small, rounded elevation on the inner surface of one end of the egg capsule is a common feature of eggs of parasitic nematodes. It has been noted in eggs of such genera as *Physaloptera*, *Diplotriaena*, *Monopetalonema*, *Dicheilonema*, *Ascaridia*, *Filaria*. In a recent study of *Filaria martis* (2) it was concluded that this structure represented the first polar body. Unfortunately, attempts to stain the material by the Feulgen process were unsuccessful although preliminary study of *Diplotriaena thomasi* by this process seemed to confirm this conclusion. It has been possible now to investigate the origin of this structure in abundant fresh specimens of *Cystidicola cristivomeri*.

Methods

The genital tracts of some specimens were removed and mounted in saline tinted with azur II under a vaseline-ringed cover slip. The genital tracts of specimens fixed in 10% formalin were studied in lactophenol tinted with cotton blue. The tracts of fresh, living specimens were removed, stuck to a slide with albumin, fixed in Carnoy's, and stained by the Feulgen process. Eggs exhibiting polar body formation, fertilization, and early cleavage were found in the seminal receptacles.

Results and Conclusions

The appearance of the eggs was essentially the same in specimens examined fresh in saline and those fixed and stained in lactophenol with cotton blue. Various stages in the formation of polar bodies and fertilization could readily be recognized. The ovum (Fig. 1) was a large, oval-shaped body with a circular nucleus and a prominent nucleolus. The cytoplasm was reticulate and often vacuolated peripherally. The egg capsule (consisting of the shell and the vitelline membrane; the latter is often difficult to discern, however) was formed immediately after penetration of the sperm into the ovum. The sperm could be seen as a rounded, refractory body towards one end of the ovum (Fig. 2). Numerous eggs were noted in which a prominent elevation had formed on the end of the ovum furthest from the resting sperm. This

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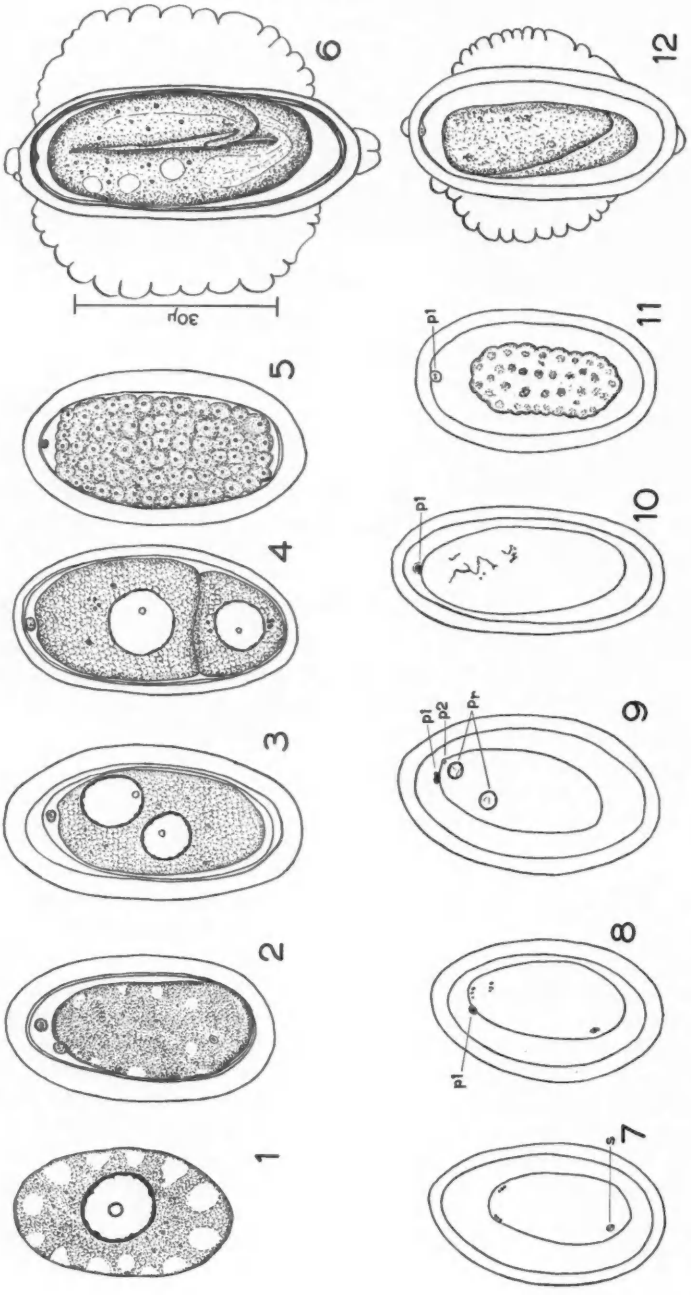
1
2
3
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5
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P1
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P1
12

structure became detached from the ovum and a further protuberance made its appearance (Fig. 2) but failed to become detached and finally disappeared. Later stages were noted in which the pronuclei had formed (Fig. 3) and numerous stages in the subsequent cleavage of eggs were followed (e.g. Figs. 4–5). In all, the first body thrown off persisted in the perivitelline space and it was finally incorporated into the vitelline membrane and is the protuberance found in fully formed eggs (Fig. 6). This structure stained blue like the ovum, embryo, and first-stage larva, in marked contrast to the egg capsule.

The main features of the production of polar bodies were easily observed in preparations stained by the Feulgen process although the technique caused the chorion, vitelline membrane, and embryo to shrink considerably. The observations on the formation of polar bodies accord with studies of numerous authors whose work has been summarized by Walton (5). Study showed clearly that the first protuberance produced and so evident in fresh material, is the first polar body; its formation is shown in Figs. 7–8. This deeply staining body became detached from the ovum but generally remained close to it during the early cleavage stages presumably because the fixing and staining had caused the vitelline membrane to shrink; it was generally impossible to see the latter. A second, smaller polar body was formed (Figs. 8–9) and could be detected in ova in which pronuclei had formed (Fig. 9). The fate of the second body was not determined but it was presumably resorbed. The first polar body was darkly stained in all the early stages (e.g. first cleavage, Fig. 10) but in later stages it generally stained only faintly (Fig. 11) and in fully formed eggs only two minute spots in its center generally took up stain (Fig. 12). Presumably the chemical composition of the first polar body gradually became altered after it had been formed.

The results seem to show that the protuberance on the inner surface of the eggs of *C. cristivomeri* is the first polar body. The structure noted on the vitelline membrane of eggs of *Ascaridia* and *Heterakis* by Dorman (4), Baylis (3), and Ackert (1) may also represent the first polar body. Ackert's figures of the eggs of *Ascaridia lineata* indicate that the protuberance is on the outside of the vitelline membrane. A study of eggs of *Ascaridia* sp. from *Bonasa umbellus* shows, however, that it is on the inside of an exceedingly delicate, vitelline membrane.

FIG. 1. Unfertilized egg. FIG. 2. Fertilized egg, first polar body in perivitelline space, second protruding from ovum. FIG. 3. Later stage showing pronuclei. FIG. 4. Two-cell stage. FIG. 5. Multicellular embryo, first polar body reduced in size. FIG. 6. Fully formed egg with first-stage larva. FIG. 7. Newly fertilized egg showing division to form first polar body. FIG. 8. Division to form second polar body, first polar body present as deeply staining body on developing ovum. FIG. 9. Ovum showing two polar bodies and pronuclei. FIG. 10. Later stage in preparation for first cleavage. FIG. 11. Multicellular embryo. First polar body persisting as elevation on egg capsule. FIG. 12. Fully formed egg.

FIGS. 1–6. Eggs fixed in 10% formalin and stained in lactophenol with cotton blue.
FIGS. 7–12. Eggs stained by Feulgen process.

ABBREVIATIONS: P1, P2 = first and second polar bodies; Pr = pronucleus; S = sperm.

References

1. ACKERT, J. E. The morphology and life cycle of the fowl nematode *Ascaridia lineata* (Schneider). Parasitology, **23**, 360–379 (1931).
2. ANDERSON, R. C. A study of *Filaria martis* Gmelin, 1790 from *Martis foina* and *Pedetes caffer*. Can. J. Zool. **38**, 157–167 (1960).
3. BAYLIS, H. A. A manual of helminthology. London. 1929.
4. DORMAN, H. P. Studies on the life cycle of *Heterakis papillosa* (Bloch). Trans. Am. Microscop. Soc. **47**, 379–413 (1928).
5. WALTON, A. C. Gametogenesis. *In* An introduction to nematology *by* Chitwood and Chitwood. Sect. II. Pt. I. Chap. 1. pp. 205–211. 1950.

BLOOD PARASITES OF BIRDS IN ALGONQUIN PARK, CANADA, AND A DISCUSSION OF THEIR TRANSMISSION[1]

G. F. Bennett and A. M. Fallis

Abstract

Fixed and stained blood smears of over 3000 birds resident in Algonquin Park, Canada, have been examined for blood parasites. Sixty per cent of the adults and 40% of the immature birds harbored one or more blood parasites. *Leucocytozoon* occurred in 60%, *Trypanosoma* in 48%, *Haemoproteus* in 26%, *Microfilaria* in 7%, and *Plasmodium* in 2% of the infected birds. High incidence and high parasitaemia of *Leucocytozoon*, *Trypanosoma*, and *Haemoproteus* coincided with the occurrence of large numbers of ornithophilic simuliids and *Culicoides* and preceded the time when most hippoboscids were recovered. Differences in incidence of parasites and the level of parasitaemia in different birds when considered in relation to the occurrence and feeding behavior of various ornithophilic flies suggest that simuliids are the vectors of *Trypanosoma*, and *Culicoides* transmit *Haemoproteus* in this area.

Introduction

The prevalence of parasites in the peripheral circulation of various birds in North America has been noted by many investigators (7, 8, 17, 18, 19, 21, 22, 23, 24, 25, 28, 30). As Manwell (24) points out, the significance of the results is sometimes obscured by the size of the samples and the lack of uniformity in reporting them. Nevertheless, the reports have shown interesting differences in the occurrence of blood parasites in different localities. However, there is a need for observations on the incidence of these blood parasites in relation to the occurrence of their probable vectors.

Surveys of blood parasites in both resident and migratory birds in Algonquin Park have been in progress for several years; a small sample of migrant birds from Point Pelee was also examined. Concurrent collections of ornithophilic blood-sucking Diptera in the area throughout the spring and summer gave qualitative information on their number, species, and feeding behavior (4). Ornithophilic simuliids, ceratopogonids, hippoboscids, and culicids have been reported as vectors of *Leucocytozoon* (10, 12), *Haemoproteus* (1, 11, 13, 27, 29), and *Trypanosoma* (3, 5, 15). Consequently, an analysis of the combined data on incidence and occurrence of biting flies should indicate the types likely to be the vectors of these blood parasites in Algonquin Park.

Materials and Methods

The survey was conducted at the Wildlife Research Station of the Ontario Department of Lands and Forests, Lake Sasagewan, Algonquin Park. Most birds, the majority of which were alive when examined, were obtained in 1957–59, although some were examined during the preceding 10 years. Blood smears made from the birds were dried and fixed in absolute alcohol, stained

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with Giemsa, and examined under low power, high power, and oil immersion until parasites were found or until 50 fields were covered. In addition, during 1958 a drop of blood was diluted in a citrated-saline (1% sodium citrate in 0.85% saline) solution and examined for trypanosomes and microfilariae. In 1957–59, Japanese mist nets were used to capture many of the birds, and after taking a blood sample, official bands were placed on each; these birds could be recognized if recaptured.

In Tables I and III, the data are presented as percentages of the total number of infected birds, rather than as percentages of the total number of birds examined. These percentages do not total 100% as many birds harbored two or more parasites, the totals of the infections with the various parasites exceeding the total number of infected birds.

Glossary of the Names of Birds Mentioned in Text

Common name	Scientific name
Grouse (ruffed)	*Bonasa umbellus*
Saw-whet owl	*Aegolius acadica*
Chimney swift	*Chaetura pelagica*
Blue jay	*Cyanocitta cristata*
Canada jay	*Perisoreus canadensis*
Crow	*Corvus brachyrhynchos*
Purple finch	*Carpodacus purpureus*
White-throated sparrow	*Zonotrichia albicollis*
Red-eyed vireo	*Vireo olivaceous*
Barn swallow	*Hirundo rustica*
Myrtle warbler	*Dendroica coronata*
Tennessee warbler	*Vermivora peregrina*
Robin	*Turdus migratorius*
Corvids	Corvidae
Woodpeckers	Picidae
Flycatchers	Tyrannidae
Ducks	Genus *Anas*
Sparrows	Fringillidae
Warblers	Parulidae
Thrushes	Turdidae

Results

The recorded incidence of blood protozoa, based on the examination of a single drop of blood, depends on the level of parasitaemia. Previous experience with *Leucocytozoon* (10) and *Haemoproteus* (1, 11, 13) showed that the peak in parasitaemia occurs relatively soon after the infection is patent. Hence the presence of small and/or many large parasites indicates a recent infection; scarcity of mature and absence of small parasites indicates a chronic infection. Consequently, differences in incidence and intensity of infections should reflect the extent to which transmission of these parasites is occurring at different times throughout the summer.

Leucocytozoon and *Trypanosoma* are the commonest blood parasites, followed by *Haemoproteus*, *Microfilaria*, and *Plasmodium* (Tables I–III). The higher incidence noted in 1957–59 (Table III) is due in part to a change in technique, namely, fresh blood was examined for *Trypanosoma* and *Microfilaria* and,

TABLE I

Incidence of blood parasites in birds, 1957–59

	Algonquin Park				Point Pelee
	Residents		Migrants		
	Adults	Immatures	Adults	Immatures	Migrants
Total birds examined	1087	1287	211	571	289
No. infected birds	696	622	78	200	82
Incidence	63%	48.5%	37%	35%	28%
No. infected birds with:					
Leucocytozoon	68% (475)*	71.5% (445)	85% (66)	84% (168)	83% (68)
Trypanosoma	44.7% (311)	29% (181)	25.6% (20)	22% (44)	11% (9)
Haemoproteus	32.8% (228)	23% (144)	1.2% (1)	8.5% (17)	28% (23)
Microfilaria	10.3% (71)	1.1% (7)	1.2% (1)	2.5% (5)	19% (16)
Plasmodium	0.8% (6)	3.3% (19)	0% (0)	0.5% (1)	6% (5)

*Figures in parentheses indicate numbers involved.

also, fewer nestling birds were included in the sample. The occurrence of the five kinds of blood parasites in the immature birds (Tables I, II) indicates that transmission occurs in the area.

Multiple infections involving two or more blood parasites were common. Of a total of 1750 infected birds from Algonquin Park, 1227 carried a single parasite, 419 carried two, 89 carried three, and 15 harbored four blood parasites. More significant is the combination of parasites in these multiple infections. *Leucocytozoon* and *Trypanosoma* occurred in the same bird 251 times, *Haemoproteus* and *Trypanosoma* in 39 birds, *Leucocytozoon* and *Haemoproteus* in 109 birds, and all three together in 39 birds.

Leucocytozoon was found in approximately 70% of the infected birds (Table I), and was more common in some species than others (Table II). For example, it was common in thrushes, especially robins, but scarce in woodpeckers; more common in the myrtle than in other warblers. It was common in sparrows and purple finches but absent in barn swallows.

Trypanosoma was found in nearly one-third of the birds examined during 1958–59 (Table III). The incidence in most preceding years, determined only by examination of dried smears, is lower and probably not indicative of the true incidence.

Haemoproteus was present in 33% of the infected adult birds and in 23% of the immatures (Table I). Some of these latter were less than 3 weeks of age when examined and, consequently, may have been too young to have parasites in the blood. Species of this parasite were especially common in woodpeckers, corvids, some sparrows, and purple finches but rare, or absent, in thrushes, warblers (except the myrtle), and the flycatchers (Table II).

Plasmodium and *Microfilaria* were seen in relatively few birds. As might be expected the latter were seen more often in adults in which the filarioids would have had time to mature and produce microfilariae. *Plasmodium* was seen more often in immature birds.

TABLE II

Incidence of blood parasites in birds in Algonquin Park, 1947–1959

	Adults							Immatures						
	Total examined	Total positive	L*	T	H	M	P	Total examined	Total positive	L	T	H	M	P
Ardeidae														
Botaurus lentiginosus	—	—	—	—	—	—	—	4	0	—	—	—	—	—
Ardea herodias	3	2	2	—	—	—	—	32	11	11	—	—	—	—
Scolopacidae														
Tringa solitaria	1	1	1	—	—	—	—	—	—	—	—	—	—	—
Philohela minor	5	0	—	—	—	—	—	—	—	—	—	—	—	—
Capella gallinago	1	0	—	—	—	—	—	—	—	—	—	—	—	—
Tetraonidae														
Bonasa umbellus	75	58	55	18	6	13	0	75	32	30	4	0	0	0
Canachites canadensis	12	9	6	3	4	5	0	3	3	3	2	0	0	0
Accipitrinae, Tytonidae, Strigidae														
Accipiter gentilis	1	0	—	—	—	—	—	6	2	2	—	—	—	—
Bubo virginianus	—	—	—	—	—	—	—	2	2	2	—	—	—	—
Circus cyaneus	2	0	—	—	—	—	—	—	—	—	—	—	—	—
Aegolius acadica	2	2	2	1	—	—	—	1	1	1	—	—	—	—
Accipiter striatus	2	2	2	—	1	—	—	1	1	1	—	—	—	—
Coccyzus erythrophthalmus	4	2	2	—	—	—	—	1	0	—	—	—	—	—
Megaceryle alcyon	2	0	—	—	—	—	—	—	—	—	—	—	—	—
Picidae														
Picoides arcticus	3	2	—	—	2	—	—	3	0	—	—	—	—	—
Dendrocopus pubescens	8	4	—	—	4	—	—	9	4	—	—	4	—	—
Dendrocopus villosus	16	15	2	—	15	—	—	8	7	1	—	6	—	—
Sphyrapicus varius	6	4	—	—	4	—	—	12	6	1	1	5	—	—
Colaptes auratus	30	30	7	2	28	—	—	65	32	6	1	29	—	—
Chaetura pelagica	7	1	—	1	—	—	—	1	0	—	—	—	—	—
Tyrannidae														
Empidonax spp.	26	8	4	5	—	—	—	28	12	4	12	—	—	—
Tyrannus tyrannus	1	0	—	—	—	—	—	7	2	2	—	—	—	—
Sayornis phoebe	1	0	—	—	—	—	—	—	—	—	—	—	—	—
Contopus virens	4	1	—	—	1	—	—	5	4	3	3	—	—	—
Empidonax flaviventris	4	0	—	—	—	—	—	6	1	—	1	—	—	—
Corvidae														
Cyanocitta cristata	24	21	17	11	13	1	—	17	14	11	1	13	1	—
Corvus corax	—	—	—	—	—	—	—	3	2	2	—	1	—	—
Perisoreus canadensis	26	6	—	4	1	—	1	20	2	—	2	—	—	—
Corvus brachyrynchos	—	—	—	—	—	—	—	10	8	8	2	5	—	—
Sturnus vulgaris	—	—	—	—	—	—	—	4	1	1	—	—	—	—

TABLE II (*Continued*)

Incidence of blood parasites in birds in Algonquin Park, 1947–1959

	Adults							Immatures						
	Total examined	Total positive	L*	T	H	M	P	Total examined	Total positive	L	T	H	M	P
Icteridae														
Quiscalus versicolor	80	58	52	6	7	16	—	24	13	13	1	—	—	—
(9–14 days of age)	—	—	—	—	—	—	—	99	8	8	—	—	—	—
Agelaius phoeniceus	3	0	—	—	—	—	—	2	2	2	—	—	—	—
Piranga olivacea	1	1	1	—	—	—	—	6	5	4	1	1	—	—
Fringillidae														
Hesperiphona vespertina	7	1	—	1	—	—	—	—	—	—	—	—	—	—
Spinus tristis	7	1	—	1	1	—	—	3	0	—	—	—	—	—
Passerina cyanea	1	1	1	—	—	—	—	3	0	—	—	—	—	—
Spinus pinus	3	0	—	—	—	—	—	—	—	—	—	—	—	—
Carpodacus purpureus	72	66	57	38	28	3	1	25	22	21	7	7	—	—
Loxia curvirostra	1	0	—	—	—	—	—	1	1	1	—	—	—	—
Pheucticus ludovicianus	11	8	6	1	3	—	—	11	9	8	3	2	—	—
Loxia leucoptera	6	4	4	2	—	2	—	—	—	—	—	—	—	—
Spizella passerina	21	16	13	10	3	—	—	35	17	15	3	7	—	—
Melospiza lincolnii	4	1	1	—	—	—	—	1	1	—	—	1	—	—
Passerculus sandwichensis	—	—	—	—	—	—	—	1	1	1	—	—	—	—
Junco hyemalis	34	15	10	8	2	—	—	25	7	5	4	4	—	—
Melospiza melodia	49	27	18	12	6	3	—	80	38	29	5	8	—	3
Melospiza georgiana	12	7	6	5	1	1	—	23	3	3	1	—	—	—
Zonotrichia leucophyrs	6	4	3	1	2	—	—	15	8	7	2	1	—	—
Zonotrichia albicollis	182	124	77	52	77	6	2	241	129	63	24	68	—	15
Pooecetes gramineus	2	1	1	—	—	—	—	18	7	5	2	2	—	—
Hirundo rustica	62	4	—	4	—	—	—	—	—	—	—	—	—	—
Bombycilla cedrorum	13	7	5	5	—	—	—	13	10	10	4	—	—	—
Vireonidae														
Vireo solitarius	3	1	1	—	—	—	—	14	5	4	1	1	—	—
Vireo philadelphicus	2	0	—	—	—	—	—	13	1	—	—	1	—	—
Vireo olivaceous	63	41	23	29	16	2	—	34	21	14	7	3	—	—
Vireo flavifrons	—	—	—	—	—	—	—	1	0	—	—	—	—	—
Parulidae														
Dendroica castanea	14	6	5	2	—	—	—	32	7	7	1	—	—	—
Mniotilta varia	21	8	4	5	—	1	—	33	3	2	2	—	—	—
Dendroica fusca	7	3	3	—	—	—	—	14	7	3	3	—	1	—
Dendroica striata	2	1	1	—	—	—	—	22	7	6	1	—	—	—
Dendroica coerulescens	9	2	1	1	—	—	—	3	0	—	—	—	—	—
Dendroica virens	4	4	4	—	—	—	—	51	13	13	4	—	—	—
Wilsonia canadensis	36	13	7	8	—	—	—	32	7	4	3	—	—	—

TABLE II (*Concluded*)

Incidence of blood parasites in birds in Algonquin Park, 1947–1959

	Adults							Immatures						
	Total examined	Total positive	L*	T	H	M	P	Total examined	Total positive	L	T	H	M	P
Parulidae—*Continued*														
Dendroica tigrina	2	1	—	1	—	—	—	9	5	5	1	—	—	—
Dendroica pennsylvanica	6	5	1	5	—	1	—	40	17	10	11	—	—	1
Dendroica magnolia	28	17	11	12	—	—	—	65	25	18	9	—	—	—
Oporornis philadelphia	5	0	—	—	—	—	—	5	1	1	—	—	—	—
Dendroica coronata	92	57	45	14	26	7	—	143	80	70	19	7	4	1
Vermivora ruficapilla	83	20	15	8	—	—	—	159	49	40	20	—	—	—
Seiurus noveboracensis	17	11	5	7	—	—	—	16	9	6	6	—	—	—
Vermivora celata	1	0	—	—	—	—	—	—	—	—	—	—	—	—
Seiurus aurocapillus	57	10	7	4	—	—	—	62	18	12	7	—	—	—
Dendroica pinus	1	1	1	—	—	—	—	—	—	—	—	—	—	—
Setophaga ruticilla	10	2	1	1	—	1	—	13	5	3	3	—	—	—
Vermivora peregrina	13	4	2	2	—	—	—	58	30	24	9	—	—	—
Wilsonia pusilla	11	6	3	3	—	—	—	13	5	3	2	—	—	—
Geothlypis trichas	32	11	2	9	—	5	—	36	7	5	3	—	—	—
Dendroica petechia	—	—	—	—	—	—	—	1	0	—	—	—	—	—
Mimidae														
Toxostoma rufum	2	2	2	2	—	1	—	—	—	—	—	—	—	—
Dumetella carolinensis	6	5	3	3	—	—	—	1	1	—	1	—	—	—
Troglodytidae														
Troglodytes aedon	2	0	—	—	—	—	—	4	0	—	—	—	—	—
Troglodytes troglodytes	5	2	—	—	—	2	—	5	0	—	—	—	—	—
Paridae, Sittidae, Certhidae														
Parus atricapillus	34	20	17	9	—	—	—	65	39	38	3	—	—	—
Certhia familiaris	2	0	—	—	—	—	—	3	0	—	—	—	—	—
Parus hudsonicus	3	1	1	—	—	—	—	16	7	5	2	1	—	—
Sitta canadensis	4	1	—	1	—	—	—	14	3	1	3	—	—	—
Sylviidae														
Regulus satrapa	10	1	—	1	—	—	—	18	4	1	3	—	—	—
Regulus calendula	6	1	—	1	—	—	—	8	3	—	3	—	—	—
Turdidae														
Hylocichla minima	12	2	2	—	—	—	—	18	5	5	—	—	—	—
Hylocichla guttata	11	6	6	—	—	—	—	19	6	6	2	—	—	—
Hylocichla ustulata	43	20	17	4	—	6	—	62	22	18	6	—	1	—
Turdus migratorius	60	50	47	17	—	11	2	66	48	47	9	—	2	3
Hylocichla fuscescens	13	9	6	5	—	—	—	24	13	12	4	—	—	—
Hylocichla mustelina	—	—	—	—	—	—	—	3	1	1	—	—	—	—

*L = *Leucocytozoon*, T = *Trypanosoma*, H = *Haemoproteus*, M = *Microfilaria*, P = *Plasmodium*.

TABLE III

Incidence of blood parasites in resident birds in Algonquin Park, 1947–1959

Year	Total examined	No. infected*	Leuc.	Tryp.	Haem.	Microf.	Plasm.
1947	42	15 (36)	15	1		2	
1948	102	26 (25)	22	6		5	
1949	103	28 (27)	22	1	3	2	1
1950	111	31 (28)	22	4	9	2	
1951	157	33 (21)	24	7	4	2	
1952	21	9 (43)	8	3			
1953	55	18 (33)	14	2		5	
1956	39	15 (39)	4	8		1	
1957	462	285 (62)	200	69	104	16	3
1958	1192	680 (57)	454	331	178	51	20
1959	720	353 (49)	166	102	88	17	2
	3004	1493 (49)	951	534	386	103	26

*Figure in parenthesis is the percentage of total birds infected.

Differences in the incidence of infection (Table I) in birds migrating south through Algonquin Park and past Point Pelee in the autumn cannot be evaluated as the previous history of these migrants is unknown. The lower incidence in birds from Point Pelee, taken about two weeks after the Algonquin migrants were examined, might be explained by assuming that chronic infections were overlooked. This is probably true of the trypanosomes, as only an 11% incidence was recorded at Point Pelee (examination of smears only).

Sixty-three per cent of the adult birds harbored blood parasites (Table I) and 48% of the immatures were infected. Only *Plasmodium* was noted in immature birds with greater frequency than in adults.

Leucocytozoon, *Trypanosoma*, and *Haemoproteus* were more prevalent among immature birds during June through July, and more birds had high parasitaemias during this same period, than in August and September (Figs. 1, 2). Consequently, transmission of these parasites must have occurred during, or just prior to, June and in July. These data are more meaningful when considered in relation to the abundance and prevalence of the blood-sucking flies of the area, some of which data will be reported later (4). These data include the total number of blood-sucking insects collected at different times throughout the summer after engorgement on ducks, grouse, crows, blue jays, robins, white-throated sparrows, and saw-whet owls. Assuming these collections indicate the numbers of these blood-sucking insects feeding on all birds in the area, these collections are used to express the relative number of ornithophilic flies feeding throughout the summer. Relatively few mosquitoes were taken, consequently the totals are omitted. Owing to differences in the number of collections of insects made on different days from a different number of birds, the number of black flies and biting midges obtained throughout the summer is expressed as the number of flies per bird per collection (Figs. 1, 2). Some simuliids and ceratopogonids are present for a longer period than illustrated, but these were too few to capture by our technique.

Two species of louse fly, namely *Ornithomyia fringillina* and *Ornithoica vicinia*, were collected from many kinds of birds in another study; their prevalence is expressed as the average number of flies per infested bird.

Most specimens of ornithophilic black flies were collected from late May until early July and most biting midges from mid-June until early July (Figs. 1, 2). No louse flies were taken until July and most were taken in August and September.

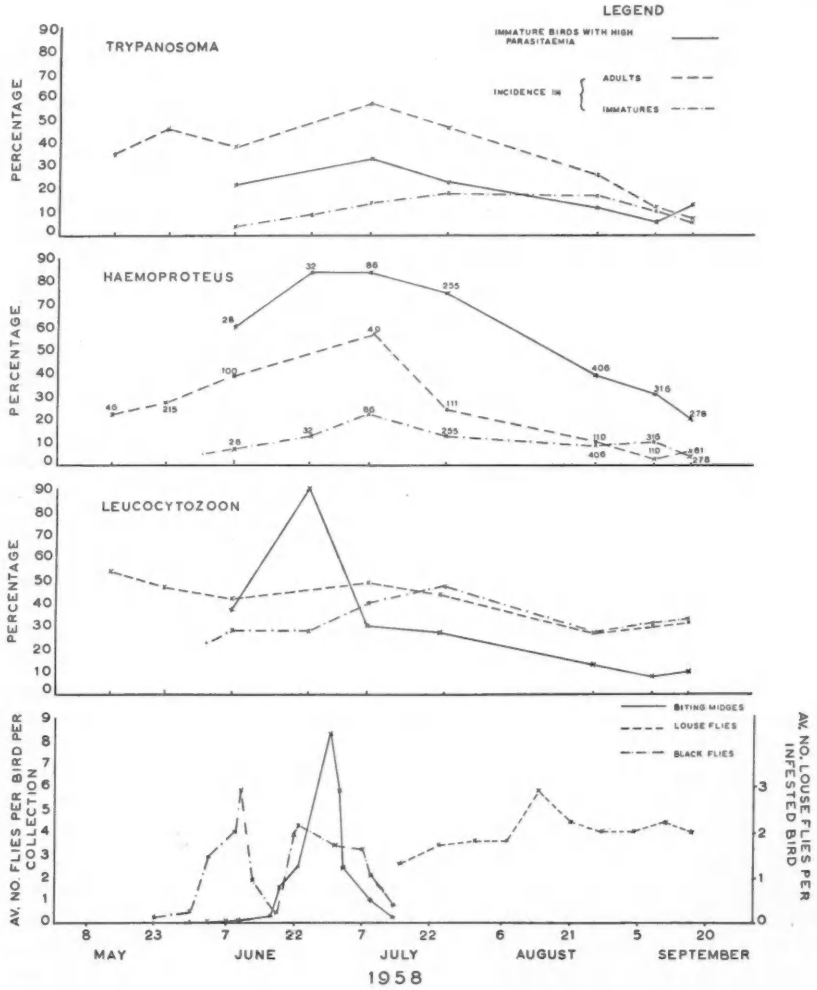

FIG. 1. Occurrence of *Leucocytozoon*, *Haemoproteus*, *Trypanosoma*, and Simuliidae, Ceratopogonidae, and Hippoboscidae throughout the summer of 1958 in Algonquin Park and the percentage of immature birds with high parasitaemias.

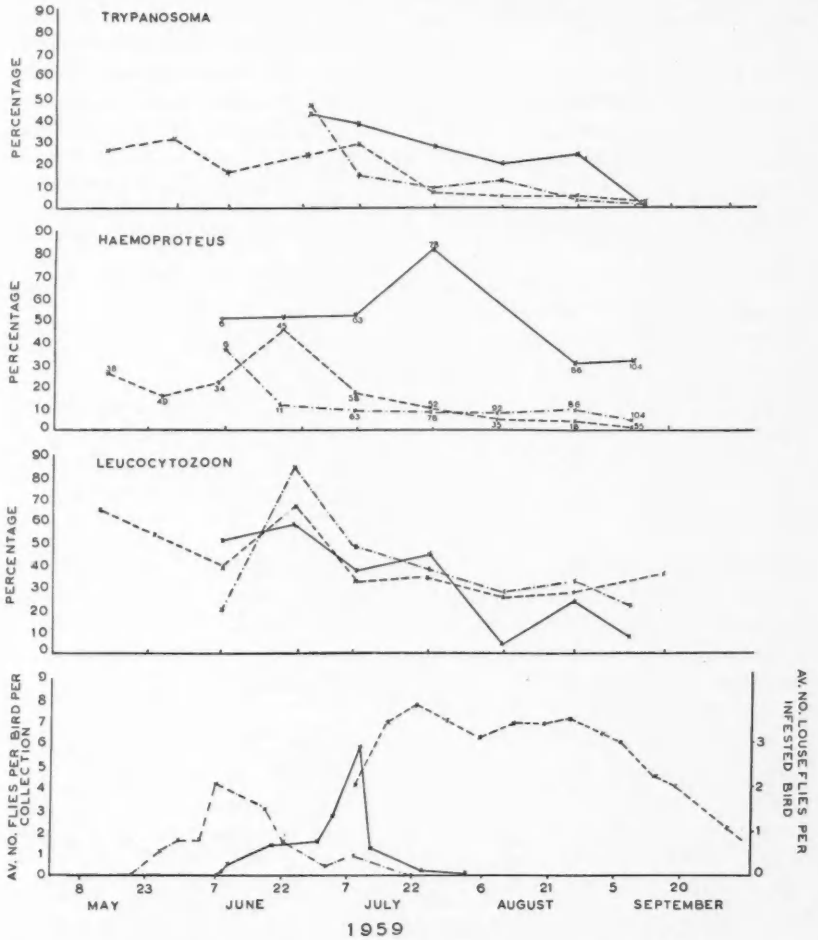

FIG. 2. Occurrence of *Leucocytozoon*, *Haemoproteus*, *Trypanosoma*, and Simuliidae, Ceratopogonidae, and Hippoboscidae throughout the summer of 1959 in Algonquin Park and the percentage of immature birds with high parasitaemias.

Oöcysts and sporozoites of *Leucocytozoon* have been demonstrated in the present study in dissections of *Simulium latipes*, *S. aureum*, *P. decemarticulatum* that had fed on infected crows and robins, and of *S. aureum* that fed on an infected owl, and of *S. latipes* that fed on an infected white-throated sparrow. Similarly sporozoites of *Haemoproteus* were found in the salivary glands of *Culicoides crepuscularis* and *C. stilobezziodes* that fed on infected crows and of *C. stilobezziodes* that fed on infected white-throated sparrows. The development of *Trypanosoma* from several birds has been noted in species of black flies, biting midges, mosquitoes, deer flies, and hippoboscids (5).

Sixty-one birds examined during one year were recaptured and examined the following year. Over 50% were carrying the same parasites noted the previous year. Thus, birds infected in one year, returning to the same locality the following year, serve as foci of infection.

Discussion

The occurrence of *Leucocytozoon*, *Trypanosoma*, *Haemoproteus*, *Microfilaria*, and *Plasmodium* in the immature birds indicates that transmission occurs in Algonquin Park.

Transmission of *Leucocytozoon* by various species of ornithophilic black flies has been clearly demonstrated (10, 12). Therefore, as expected, active transmission of *Leucocytozoon* occurs at that period when ornithophilic simuliids are prevalent (Figs. 1, 2).

Data pertaining to the transmission of *Trypanosoma* are more difficult to interpret. Previous reports have indicated that hippoboscid flies (3) and mosquitoes (15) are vectors. In a concurrent study (5), it was shown that flagellates will develop in, and transmission can be produced by using, black flies, biting midges, mosquitoes, and hippoboscids. The incidence and level of parasitaemia of trypanosomes throughout the summer (Figs. 1, 2) indicates that active transmission occurred during mid-June through July. This is coincident with the peak in numbers of black flies and biting midges. Lower levels of parasitaemia are noted in August and September, the period when hippoboscids were most prevalent. *Leucocytozoon* and trypanosomes occurred together more frequently than any other two parasites, suggesting that they have a common vector.

Ornithophilic simuliids are probably the important vectors of avian trypanosomes in Algonquin Park. Other evidence (5) supports this conclusion. Probably simuliids are the important vectors elsewhere. Mohammed (26) noted a low incidence of *Leucocytozoon* and *Trypanosoma* in birds in lower Egypt where Simuliidae are reported scarce or absent (14); in contrast, *Haemoproteus* and *Plasmodium* were common (26). Jordan (22) and Love *et al.* (23) working in Georgia found *Leucocytozoon* in only 4 birds, and trypanosomes in 14, compared to over 300 with *Haemoproteus* and 300 with *Plasmodium*. Gaud and Petitot (reported by Mohammed (26)) found few birds with *Leucocytozoon* and *Trypanosoma* in Morocco although *Haemoproteus* and *Plasmodium* were common. It is interesting to note that Duke and Robertson (9) in 1912 suggested simuliids as vectors of avian trypanosomes in Uganda. Although simuliids are probably the important vectors, other reports (3, 5, 15) indicate other blood-sucking Diptera can transmit these parasites.

Transmission of *Haemoproteus* by Hippoboscidae has been reported (1, 27, 29) although other investigators (16, 20, 24) have suspected that hippoboscids are not the only vectors of this parasite. Recently *Culicoides* were demonstrated (11, 13) as intermediate hosts for species of *Haemoproteus*.

A high incidence and high level of parasitaemia, denoting active transmission, was observed in birds examined during June and July (Figs. 1, 2), coincident with the peak numbers of *Culicoides*. Chronic infections predominated during August and September, the period when hippoboscids were abundant. Dissection of biting midges fed on birds infected with *Haemoproteus* demonstrated developing stages of the parasite. In addition, although purple finches were commonly infected with *Haemoproteus* (Table II), hippoboscids were not collected from them; conversely, although hippoboscids were frequent parasites of thrushes, no *Haemoproteus* was found in these birds (Table II). These data indicate that *Culicoides*, not hippoboscids, are the vectors of *Haemoproteus* in Algonquin Park. Further investigations of the epizootiology of infections with *Haemoproteus* in different birds will be necessary to explain the incidence noted in various species. For example, *Haemoproteus* was not found in thrushes (Table II) although Manwell (25) reported it from robins in New York State. *Haemoproteus* was common in the red-eyed vireo (Table II) and in the myrtle warbler but absent in the other vireos and warblers.

Plasmodium was found rarely compared to its occurrence elsewhere (22, 23). Although many species of mosquitoes occur in Algonquin Park, few have been captured, following engorgement, on birds (4).

The scarcity of microfilariae in immature birds is to be expected as probably many were examined before filarioids developing in them produced microfilariae. Moreover, as Manwell (24) points out, if filarioids show a periodicity, infections will be overlooked unless blood is examined at the right time. Hence the incidence reported herein for microfilaria is certainly minimal. The incidence of microfilaria is higher than that of *Plasmodium* and this, taken with the fact that few mosquitoes were feeding on birds, suggests some other vector. Anderson (2) demonstrated the development of an avian filarioid in an ornithophilic simuliid. Probably, simuliids are the vectors of many of these nematodes.

Attention has been drawn to the higher incidence of blood parasites in adult birds (Tables I, II). As active transmission occurs during June and July, conceivably, many immature birds examined in August and September were hatched after the period of peak transmission. Immature birds examined during June and July may have been examined before their infections were patent. On the other hand, adult birds have been exposed to at least one season of transmission. It is not surprising, therefore, that the incidence in adult birds is higher.

The common occurrence of *Leucocytozoon* and *Haemoproteus* in adult birds in May and early June (Figs. 1, 2) could be the result of relapse and/or acquisition of new infection during migration north. Chernin's (6) observations on relapses in *L. simondi* and our own unpublished data on this species and on *H. nettionis* show that parasitaemia following relapse is low compared to that following initial infection. Relatively high parasitaemias of *Leucocytozoon* and *Haemoproteus* were noted in adult birds in late May, suggesting that many birds acquired a new infection during their migration north.

Attention has been drawn to the differences in incidence of blood parasites among various species and groups of birds (Table II). Some differences may result from differences in the feeding behavior of various ornithophilic flies (4). One notes the high incidence of all blood parasites in the blue jay and the low incidence in Canada jays (Table II). About five times as many flies fed on blue jays as on Canada jays (4); this may explain the high incidence in the former birds. Similarly, *Leucocytozoon* is common in thrushes but scarce in woodpeckers (Table II), and more simuliids fed on robins than on woodpeckers (4). Significant differences also occur in the incidence of *Leucocytozoon* in related birds; cf. robins and other thrushes, myrtle and Tennessee warblers to other warblers (Table II). Perhaps it is more than coincidence that birds with the highest incidence of *Leucocytozoon* usually nest at heights five feet or more above the ground, at which level many woodland species of ornithophilic simuliids are known to feed (4). Many of the birds with low incidence nest on or near the ground. Blood parasites were rarely found in the barn swallow and chimney swift (Table II). The nesting and roosting habits of these birds probably reduce to a minimum their chances of being bitten by blood-sucking Diptera.

Acknowledgments

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References

1. ADIE, H. A. The sporogony of *Haemoproteus columbae*. Indian J. Med. Research, **2**, 671 (1924).
2. ANDERSON, R. C. The life cycle and seasonal transmission of *Ornithofilaria fallisensis* Anderson, a parasite of domestic and wild duck. Can. J. Zool. **34**, 485–525 (1956).
3. BAKER, J. R. Studies on *Trypanosoma avium*. Danilewsky 1885. II. Transmission by *Ornithomyia avicularia* L. Parasitology, **46**, 321–334 (1956).
4. BENNETT, G. F. On some ornithophilic blood-sucking Diptera in Algonquin Park, Ontario Canada. Can. J. Zool. **38**, 377–389 (1960).
5. BENNETT, G. F. On the transmission and specificity of some avian trypanosomes. Unpublished.
6. CHERNIN, E. The relapse phenomenon in the *Leucocytozoon simondi* infections of the domestic duck. Am. J. Hyg. **56** (2), 101–118 (1952).
7. CLARKE, C. H. D. Some records of blood parasites from Ontario birds. Can. Field-Naturalist, **60** (2), 34 (1946).
8. COATNEY, G. R. A check-list and host-index of the genus *Haemoproteus*. J. Parasitol. **23**, 88–105 (1936).
9. DUKE, H. L. and ROBERTSON, M. Observations on fowls and ducks in Uganda with relation to *Trypanosoma gallinarum* and *T. gambiense*. Proc. Roy. Soc. B, London, **85**, 378–384 (1912).
10. FALLIS, A. M., ANDERSON, R. C., and BENNETT, G. F. Further observations on the transmission and development of *Leucocytozoon simondi*. Can. J. Zool. **34**, 389–404 (1956).
11. FALLIS, A. M. and WOOD, D. M. Biting midges (Diptera:Ceratopogonidae) as intermediate hosts for *Haemoproteus* in ducks. Can. J. Zool. **35**, 425–435 (1957).
12. FALLIS, A. M. and BENNETT, G. F. Transmission of *Leucocytozoon bonasae* Clarke to ruffed grouse (*Bonasa umbellus* L.) by the black flies *Simulium latipes* Mg. and *Simulium aureum* Fries. Can. J. Zool. **36**, 533–539 (1958).

13. FALLIS, A. M. and BENNETT, G. F. Description of *Haemoproteus canachites* n.sp. (Sporozoa: Haemoproteidae) and sporogony in *Culicoides* (Diptera:Ceratopogonidae). To be published.
14. GARNHAM, P. C. C. Distribution of blood protozoa in Africa. Proc. Linnean Soc. London, **165** (1), 61–66 (1954).
15. GREWAL, M. S., DAVID, A., and CHOWDHARY, D. S. Preliminary report on the further studies on the "so-called" natural cryptic (occult) trypanosome of the house sparrow *Passer domesticus* Linnaeus. Indian J. Malariology, **11** (4), 415–418 (1957).
16. HANSON, H. C., LEVINE, N. D., KOSSACK, C. W., KANTOS, S., and STANNARD, L. J. Parasites of the mourning dove (*Zenaidura macroura carolinensis*) in Illinois. J. Parasitol. **43** (2), 186–193 (1957).
17. HERMAN, C. M. The blood protozoa of North American birds. Bird Banding, **15** (8), 89–112 (1944).
18. HERMAN, C. M. Blood protozoa in Cape Cod birds. Trans. Am. Microscop. Soc. **57**, 132–141 (1938).
19. HERMS, W. B., KADNER, C. G., GALINDO, P. V., and ARMSTRONG, D. F. Blood parasites of California birds. J. Parasitol. **25**, 511–512 (1939).
20. HUFF, C. G. Studies on *Haemoproteus* of mourning doves. Am. J. Hyg. **16**, 618–623 (1932).
21. HUFF, C. G. A survey of blood parasites of birds caught for banding purposes. J. Am. Vet. Med. Assoc. **90**, 615–620 (1939).
22. JORDAN, H. B. Blood protozoa of birds trapped at Athens, Georgia. J. Parasitol. **29**, 260–263 (1943).
23. LOVE, G. L., WILKIN, S. A., and GOODWIN, M. H. Incidence of blood parasites in birds collected in south-west Georgia. J. Parasitol. **39**, 52–57 (1953).
24. MANWELL, R. D. The blood protozoa of seventeen species of sparrows and other Fringillidae. J. Protozool. **2**, 21–27 (1955).
25. MANWELL, R. D. Relative incidence of blood parasites in robins of Central New York and of the High Rockies. J. Protozool. **2**, 85–88 (1955).
26. MOHAMMED, A. H. H. Systematic and experimental studies on protozoal blood parasites of Egyptian birds. Vol. I. Cairo Univ. Press. 1958.
27. O'ROKE, E. C. The morphology, transmission, and life history of *Haemoproteus lophortyx* O'Roke, a blood parasite of the California Valley quail. Univ. Calif. Publs. Zool. **36** (1), 1–50 (1930).
28. ROBINSON, E. J. Observations on the epizootiology of filarial infections in two species of the avian family Corvidae. J. Parasitol. **41** (2), 209–214 (1955).
29. TARSHIS, B. The transmission of *Haemoproteus lophortyx* O'Roke of the California quail by hippoboscid flies of the species *Stilbometopa impressa* (Bigot) and *Lynchia hirsuta* Ferris. Exptl. Parasitol. **4** (5), 464–491 (1955).
30. WIRTH, W. W. Blood parasites of Louisiana birds. Proc. Louisiana Acad. Sci. **8**, 77–82 (1944).

STUDIES ON THE TRANSAMINASE ACTIVITY OF MUSCLE TISSUE FROM ALLATECTOMIZED ROACHES, PERIPLANETA AMERICANA[1]

SHU-YI WANG AND S. E. DIXON

Abstract

The transaminase activity of roach muscle homogenate was measured by the spectrophotometric method for oxalacetic acid. The transaminase activity of muscle from allatectomized roaches of both sexes of adults and nymphs was significantly lower than in normal animals. There was also a significant difference between sexes. The reduction in transaminase activity of muscle from female adults was about twice that of male adults but between nymphs the difference due to sex was not nearly so striking. The activity of muscle tissue due to allatectomy was considerably more reduced in male nymphs than in male adults. The significance of these results is discussed.

Introduction

Wigglesworth (10, 11) has reviewed the work done on the role of the corpora allata in insects since the time he first demonstrated in 1934 that they were endocrine glands (9). The corpora allata control postembryonic development by acting in conjunction with the prothoracic gland hormone to induce larval molts. They control the development of ovarioles in hemimetabolous insects. Their removal gives rise to increased fat and carbohydrate stores but decreased protein synthesis. They also have a general effect on the metabolism of cockroach muscle manifest by increased oxygen consumption after allatectomy.

Recent work (3, 4, 12) suggests that in Lepidoptera another function of the corpora allata is that of activating the prothoracic glands.

This study provides information on the role of the corpus allatum in regulating amino acid metabolism.

Methods and Materials

Experimental Animals

The experiments were performed on roaches which had been reared at 28° C in glass jars and supplied with Master dog kibbles and water.

Surgical Procedure

The corpora allata of *Periplaneta americana* are small, spherical glands which lie in the back of the head a short distance behind the brain, one on either side of the oesophagus. Each gland is connected anteriorly by a nerve to the corpus cardiacum. The corpora cardiaca in turn have nervous connections with the brain.

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The corpora allata were removed by a procedure similar to that described by Bodenstein (1). Excised glands were checked by microscopic examination to make sure that they were intact. Control roaches were those which underwent a sham operation in which the corpora allata were left intact.

This study involved allatectomized females, males, and nymphs of both sexes which were kept for varying periods after the operation.

Preparation of Homogenate

Roaches were chilled before dissection to make possible cleaner and more rapid removal of tissue relatively free of fat.

After decapitation of the specimens, the thoracic muscles were dissected, and pooled samples of four or five roaches were homogenized at a concentration of 1.5% fresh weight of tissue in phosphate buffer (0.05 *M*, pH 7.4) centrifuged to remove cuticle and debris. The fatty layer was removed by decantation and filtration. Until the transaminase reactions were carried out the homogenate was kept in a container surrounded by crushed ice. Muscle homogenates were prepared from allatectomized and control roaches.

Chemical Methods

Micro determinations of nitrogen were based on the method of Cole *et al.* (2) using aliquots of the homogenates used in the transaminase reactions.

Transaminase determinations were performed in a manner similar to those described by Kilby and Neville (5) where oxalacetic acid is formed as the product of transamination and measured spectrophotometrically.

Assay Procedure

To the quartz cell of a Beckman spectrophotometer were added 0.3 ml of tissue homogenate prepared as described above and 1.0 ml of buffered pyridoxal phosphate (0.7 micromole per ml). The mixture was incubated for 10 minutes at 37° C and then 1.0 ml of buffered aspartate (20 micromoles per ml) was added. This mixture was further incubated for 10 minutes at 37° C after which 0.2 ml of buffered α-ketoglutarate (100 micromoles per ml) was added. The blanks were complete systems from which both aspartate and α-ketoglutarate were omitted. The blanks were run with the test system and read at 280 mμ at 5-minute intervals. The results are expressed as oxalacetate (moles $\times 10^{-6}$) formed per mg nitrogen against time.

Results

Transaminase Activity of Muscle Homogenates from Allatectomized Adult Females

The transaminase activity of allatectomized females is shown in Fig 1. Each point on the curve is the average of three groups with four insects in each group. The averages for the early periods in Figs. 1 and 3 do not go through the origin and these have therefore been indicated by a broken line. Determinations were made 50 days after the operation. The transaminase activity of muscle homogenate from allatectomized roaches is seen to be much lower than that of normal roaches.

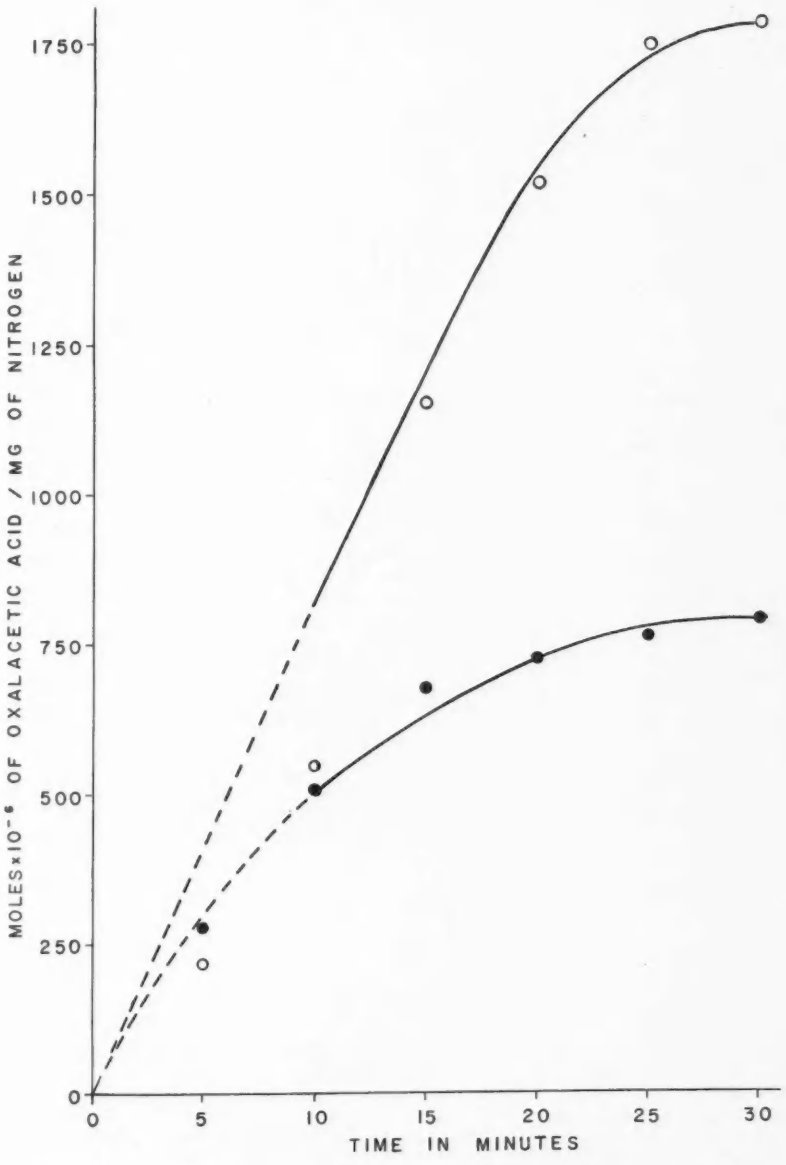

FIG. 1. Oxalacetic acid formed by muscle tissue homogenates from allatectomized (●) and control (○) female adult roaches.

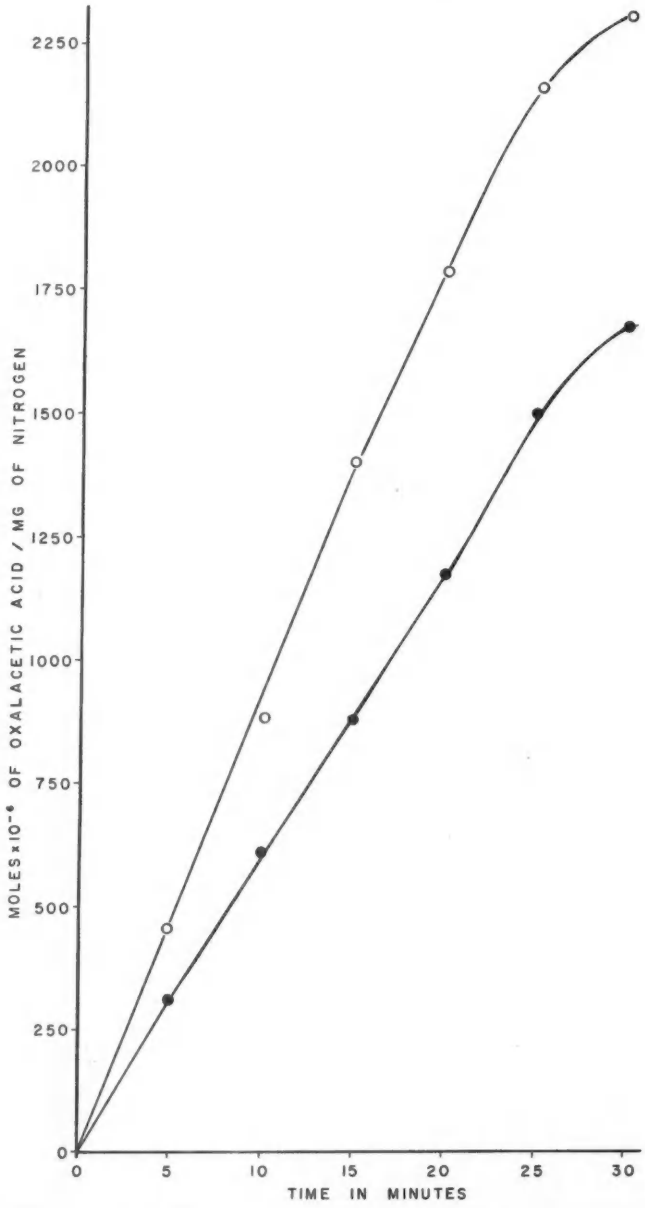

FIG. 2. Oxalacetic acid formed by muscle tissue homogenates from allatectomized (●) and control (○) male adult roaches.

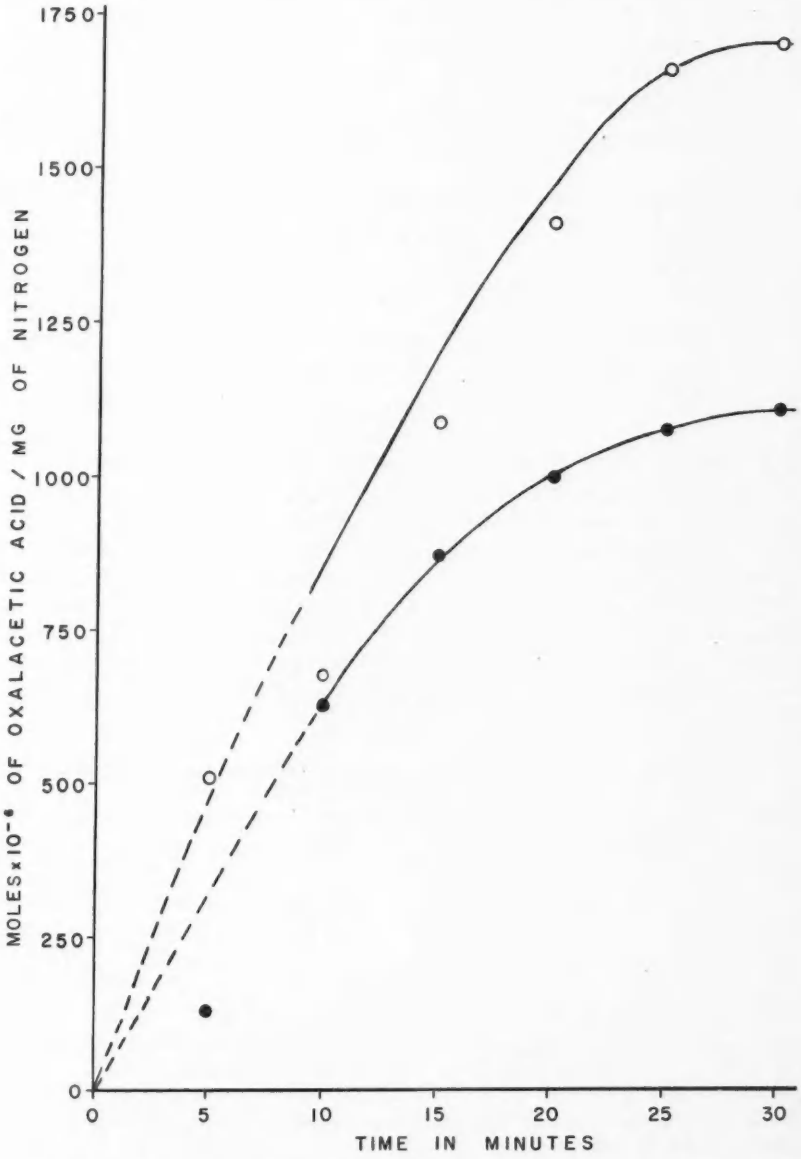

FIG. 3. Oxalacetic acid formed by muscle tissue homogenates from allatectomized (●) and control (○) last instar female nymphal roaches.

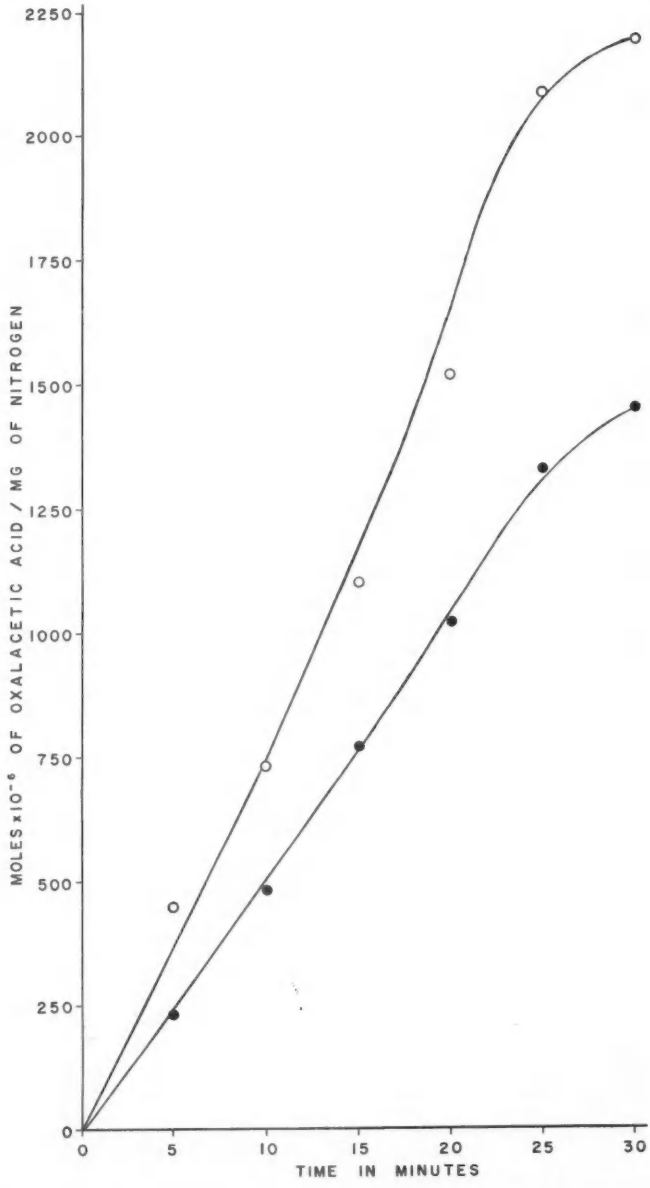

FIG. 4. Oxalacetic acid formed by muscle tissue homogenates from allatectomized (●) and control (○) last instar male nymphal roaches.

Transaminase Activity of Muscle Homogenate from Allatectomized Adult Males

The transaminase activity was determined on four groups of four roaches 75 days after allatectomy and the results compared in Fig 2. It will be seen that allatectomy has a similar effect on males as on females although the transaminase activity is not reduced to nearly the same extent.

Transaminase Activity of Muscle Homogenate from Allatectomized Nymphs of Both Sexes

The transaminase activity of muscle homogenate was determined 90 days after allatectomy on 15 last-instar female nymphs and 13 last-instar male nymphs. Figure 3 represents the data from three groups of five female nymphs. Figure 4 represents the data from one group of five and two groups of four male nymphs.

An inspection of both curves indicates again a noticeable reduction in activity after allatectomy in the nymphal stage for both sexes.

Discussion and Conclusions

Allatectomy results in lowered transaminase activity of muscle homogenate in both sexes during the adult stage and during the last nymphal stage.

Table I shows the difference between operation and sex for the adults and indicates that not only is there a difference due to the operation but also to sex. The periods between operation and transaminase determinations were convenient and though they varied from 50 days for females to 90 days for nymphs we attach no significant importance to this variation. The reduction in the transaminase activity of allatectomized female muscle tissue homogenate was about twice that of allatectomized male muscle tissue homogenate.

TABLE I

Averages and differences between operation and sex of adults of oxalacetic acid (moles $\times 10^{-6}$) production based on a 30-minute period

	Female	Male	Average	Difference
Allatectomized	789 ± 129*	1831 ± 129	1310 ± 79	1042
Control	1780 ± 129	2270 ± 129	2025 ± 79	490
Average	1284 ± 79	2051 ± 79		
Difference	991	439		

* Standard error.

TABLE II

Averages and differences between operation and sex of last-instar nymphs of oxalacetic acid (moles $\times 10^{-6}$) production based on a 30-minute period

	Female	Male	Average	Difference
Allatectomized	1105 ± 151*	1452 ± 151	1279 ± 107	347
Control	1696 ± 151	2197 ± 151	1947 ± 107	501
Average	1401 ± 107	1825 ± 107		
Difference	591	745		

* Standard error

Table II shows a significant difference in the transaminase activity of muscle homogenate from nymphs subjected to allatectomy and also a significant difference between sexes although the latter difference is less striking than the difference between adult members of the sexes.

These data also show apparent differences in activity between adult and nymphal males, the reduction in activity due to the operation being greater in the nymphs.

These data are interesting when compared with the results of allatectomy reported elsewhere in the literature. Pfeiffer (7) suggested that the corpora allata influenced the mobilization of proteinaceous material during egg production in *Melanoplus*. Castration gave rise to increased free amino acids in the blood. Allatectomy gave rise to increased fat mobilization with little if any protein synthesis. Bodenstein (1) reported a similar increased fat production in allatectomized *Periplaneta*. Scharrer (8) has demonstrated that allatectomy in the roach *Leucophaea* resulted in increased glycogen as well as increased fat stores in the fat bodies, but egg production failed to occur. Mulkern and Lawson (6) have shown that the corpora allata are not necessary for sperm production by male roaches. The data presented here indicate that the corpora allata are nevertheless essential in males and that they are involved in amino acid metabolism. The effect of allatectomy in transamination in adult males is less striking but still apparent. In female roaches, in which the corpora allata are necessary for egg production, transamination is greatly reduced. In nymphs, in which these glands are necessary for molting, the transamination is reduced the most. Perhaps then, the regulation of amino acid metabolism, particularly transamination, is one direct influence which the corpora allata exert at all stages, this influence being most obvious where protein synthesis is greatest, namely in females and nymphs.

Acknowledgments

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References

1. Bodenstein, D. Studies on the humoral mechanisms in growth and metamorphosis of the cockroach, *Periplaneta americana*. II. Function of the prothoracic gland and corpora cardiacum. J. Exptl. Zool. **123**, 413–431 (1953).
2. Cole, J. O. and Parks, C. R. Semi-microkjeldahl procedures for control laboratories. Ind. Eng. Chem. Anal. Ed. **18**, 61 (1946).
3. Gilbert, L. I. and Schneiderman, H. A. Prothoracic gland stimulation by juvenile hormone extracts of insects. Nature, **184**, 171–173 (1959).
4. Ichikawa, M. and Nishitsutsuji-Uwo, J. Studies on the role of the corpus allatum in the Eri-silkworm *Philosamia Cynthinia* Ricini. Biol. Bull. **116**, 88–94 (1959).
5. Kilby, B. A. and Neville, E. Amino acid metabolism in locust tissues. J. Exptl. Biol. **34**, 276–289 (1957).
6. Mulkern, G. B. and Lawson, F. A. Some factors affecting the male reproductive system in cockroaches (Orthoptera, Blattidae). J. Kansas Entomol. Soc. **30**, 54–57 (1957).

7. PFEIFFER, I. W. Experimental study of the function of the corpora allata in the grasshopper *Melanoplus differentialis*. J. Exptl. Zool. **82**, 439–461 (1939).
8. SCHARRER, B. The corpus allatum of *Leucophaea maderae* (Blattaria). Proc. Xth Intern. Congr. Entomol. **2**, 57 (1956).
9. WIGGLESWORTH, V. B. The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and "metamorphosis". Quart. J. Microscop. Sci. **77**, 191–222 (1934).
10. WIGGLESWORTH, V. B. Physiology of insect metamorphosis. Monographs in experimental biol. No. 1. Cambridge Univ. Press. 1954.
11. WIGGLESWORTH, V. B. The action of growth hormones in insects. Symposium Soc. Exptl. Biol. **11**, 204–227 (1957).
12. WILLIAMS, C. M. The juvenile hormone. 1. Endocrine activity of the corpora allata of the adult Cecropia silkworm. Biol. Bull. **116**, 323–338 (1959).

EFFECTS OF SPRAYING 2,4-D AMINE ON COCCINELLID LARVAE[1]

JEAN B. ADAMS[2]

Abstract

When coccinellid larvae in six different age groups were sprayed with 2,4-D amine and then confined in glass vials, two main effects were seen: first, mortality was increased four times in all age groups; and secondly, the mean time to pupation increased in all age groups except the 1-day-old larvae.

Introduction

Widespread reports of abnormal numbers of aphids on grain in 1955 throughout Canada (5) brought attention to these pests. Extensive and costly insecticidal controls appeared to limit the pests although many acres of barley were reportedly destroyed. In 1956, T. C. Chiasson,[3] C. F. Everett,[4] M. E. MacGillivray,[5] and I examined grain fields in New Brunswick to determine the aphid conditions in fields not treated with insecticides. Among our observations we noted that early in the aphid infestation coccinellid adults and larvae became numerous and active (unpublished data). In 1958 again with the co-operation of Mr. Chiasson I examined grain fields to which herbicide had been applied. Coccinellid larvae collected from these areas died soon after collection but larvae handled in a similar manner from untreated fields survived. To explore the possible detrimental effect of herbicides on coccinellid larvae, I made the following investigation in 1959.

Material and Methods

Adult coccinellid beetles of three species (*Coccinella transversoguttata* Fald., *Hippodamia tredecimpunctata* (L.), and *Coccinella perplexa* Muls.) were collected from plots of oats on the Lincoln substation, Fredericton, during the first week in July. By far the greatest number of the beetles were of the species *Coccinella transversoguttata* Fald. The insects were sexed and mated. The females were then placed in glass vials, 3 in. × ¾ in., with cotton wool stoppers; strips of blotting paper were placed in the vials and most of the eggs were laid on these; the beetles were fed living aphids daily to satiation.

Due to the rabid cannibalism of the insects, egg masses (usually of 20 to 30 eggs) were removed as soon as possible. As with the adults it was necessary to isolate the young almost as soon as they hatched in order to minimize cannibalism. In this connection we noted that larval mortality was high if the young were removed before they had consumed the egg cases from which

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they had emerged. We handled all larvae with squirrel hair brushes and took particular care to do as little damage as possible.

To feed the larvae we used about 200,000 living aphids. During the first instar, which lasted about four days, a developing larva consumed fewer than 5 aphids per day; during the second and the third instars, each of about two days, the demand increased to 10 aphids per day; and in the final instar of about five days, the larva ate up to 25 aphids per day. We fed the larvae daily to satiation.

Treatments of 2,4-D amine[6] and water were applied to larvae in six age groups—a mixture of ages, and 1, 3, 6, 9, and 12 days. The 2,4-D amine was applied with a hand atomizer calibrated to apply spray at the rate of 8 oz of acid equivalent per acre. We examined 330 larvae; of these, 227 were treated with 2,4-D and 103 were reared unsprayed as controls. After treatment each insect was returned to the vial, and thence examined daily until death or emergence as an adult beetle. At each examination debris was removed from the vials and the larvae were given fresh food; obvious deformities were recorded. All tests were conducted in an insectary on the substation.

Results

In the first test in late July and early August with larvae in a mixed age group, 77 insects were sprayed with 2,4-D amine and 73 were left unsprayed as controls. Thirty-one of the sprayed larvae died before pupation compared to eight of the unsprayed insects. Larvae that survived developed at the same rate in both treatments.

TABLE I

The effect of spraying 2,4-D amine on coccinellid larvae of different ages in August and September, 1959 (based on 30 insects in each test)

	Checks (unsprayed larvae)	Age, in days, of sprayed larvae				
		1	3	6	9	12
Mean days to pupation	16	15	21	23	25	27
Mean days to maturity	20	20	30	28	29	35
% that died before pupating	7	27	40	43	50	57
% that died by end of experiment (larvae and pupae)	20	47	47	60	60	70

To determine if age of larvae made any difference to the effects of 2,4-D as noted in the mixed age group, we studied its effects on coccinellids in five separate age groups from the same egg assembly. These tests were started on August 22 and carried on until September 26. The results (Table I) show that there may be two kinds of effect, one on the rate of development and another on mortality. In the 1-day-old age group, as in the preliminary tests, we found that the development of treated and untreated larvae was the same;

[6]The 2,4-D amine (mixed amine salts of dichlorophenoxyacetic acid), brand name "Amsol" was purchased from Niagara Brand Chemical Company, Burlington, Ontario, and supplied for this experiment through the courtesy of the Field Crops Section, Research Station, Fredericton, N. B.

however, there was a lengthening of the development period of larvae treated when they were 3, 6, 9, and 12 days old. The effect on mortality in this test was essentially the same as found in the mixed age group. It was more than twice as great in the 2,4-D-treated larvae as in the untreated controls up to the time of pupation. Mortality during pupation was no greater in the sprayed than in the unsprayed groups.

Larvae whose development had been lengthened as a result of the 2,4-D sprays finally matured in the latter part of September. An examination of the temperatures that prevailed during this period showed that they were unusually low between September 14 and 18. These low temperatures may have contributed to the further lengthening of the development period and to the mortality of these larvae beyond that attributable to the 2,4-D sprays alone.

Records and observations of individual insects showed that deformity was more prevalent when larvae were sprayed in the later stages of their development; this might be explained on the basis that similarly affected younger stages died and the extent of deformity could not be determined. When they were treated at an early age, surviving treated larvae were often smaller as they approached maturity than untreated specimens at the same stage of development.

Discussion

In New Brunswick, herbicides for weed control in grain are usually applied when the crop is from 6 to 8 in. high. Studies on aphid development in grain fields not sprayed with herbicide show that aphids are present from the time grain is 2½ in. high until it reaches a height of 30 in. (1). Closely associated with the appearance of these aphids is the presence and activity of their coccinellid predators. The first coccinellids noted have been adult beetles; the larval stage follows within 2 weeks. A slight delay in the application of the herbicide could make it coincide with the presence of coccinellid larvae with the lethal effects noted in this study. Such a delay is not uncommon since the application of herbicides, like most field practices, is dependent on favorable weather.

Aphid damage has been most generally reported from "late seeded grains". It is not known whether these areas were also late in receiving herbicide treatment but it seems likely. A setback such as we have found associated with the application of herbicides to coccinellid larvae gives the aphid population an unnatural advantage; this may account in part for a later season "upsurge" in population of the aphids thus freed from half of the early coccinellid predation.

In 1956 Ripper (7) reviewed in some detail the "effect of pesticides on balance of arthropod populations". By "pesticides" he referred to insecticides and fungicides; the effect of herbicides was not considered. The review emphasized, as have the studies of Pickett, Lord, and others (6, 4, 2), the disturbances of the balance of populations which can result from the continuous

and widespread use of chemicals as pest controls. Ripper also discussed the evidence that a pesticide could act either as a stimulant to a secondary pest or as a lethal factor, as it is in our case on the population of natural enemies, thus making possible a "resurgence" of insect populations within a treated area (8).

Carlson (3), in a study of the insecticidal control for lygus bugs, makes a statement that has equal application to herbicide practices when he says "data from field trials... showed that it was advisable to evaluate new treatments in terms of their effect on beneficial insects as well as on lygus bugs". Herbicides should be applied with the same care so that beneficial insects may not be sacrificed and pest conditions aggravated instead of improved.

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References

1. ADAMS, J. B. and MACGILLIVRAY, M. E. Laboratory and field development of an oat infesting aphid in New Brunswick. To be published.
2. BARTLETT, B. R. Laboratory studies on selective aphicides favoring natural enemies of the spotted alfalfa aphid. J. Econ. Entomol. **51**, 374–378 (1958).
3. CARLSON, E. C. Evaluation of insecticides for lygus bug control and their effect on predators and pollinators. J. Econ. Entomol. **52**, 461–466 (1959).
4. LORD, F. T. The influence of spray programs on the fauna of apple orchards in Nova Scotia. III. Mites and their predators. Can. Entomologist, **81**, 202–230 (1949).
5. MACNAY, G. *In* Field crop insect section of the Canadian Insect Pest Review—Summary, **33**, 344–346 (1955).
6. PICKETT, A. D. A critique on insect chemical control methods. Can. Entomologist, **81**, 67–76 (1949).
7. RIPPER, W. E. Effect of pesticides on balance of arthropod populations. Ann. Rev. Entomol. **11**, 403–438 (1956).
8. RIPPER, W. E., GREENSLADE, R. M., and HARTLEY, G. P. A new systemic insecticide Bis (Bis dimethylamine phosphonous) anhydride. Bull. Entomol. Research, **40**, 481–501 (1950).

QUALITATIVE CHANGES IN NATURAL POPULATIONS DURING CHANGES IN ABUNDANCE[1]

W. G. WELLINGTON[2]

Abstract

This is an extension of earlier studies of the effects of individual differences on population dynamics. The test species is the western tent caterpillar, *Malacosoma pluviale* (Dyar), which exhibited qualitative changes in its collapsing population on southern Vancouver Island between 1956 and 1959.

There are preliminary descriptions of recently established categories of individuals differing in total activity and behavior, and of the biological consequences of mixing different proportions of these active and sluggish larvae in colonies. A colony with enough active individuals built several long, clavate tents, dispersed them widely, and foraged far away from them. A colony with many sluggish individuals seldom constructed more than one compact, pyramidal tent, and fed nearby. Sluggish colonies were less viable than active colonies in harsh environments, and some were too sluggish to survive in the most favorable circumstances.

Between 1956 and 1959, fourth-instar colonies along 156 miles of roadways in the outbreak area decreased from 74,000 to 251. In any infestation, the proportion of active colonies declined from nearly 100% to 45–55% as years of residence in the locality increased from 1 to 4. Thereafter, the proportion of active colonies rose again as those too sluggish to survive began to disappear from the population. The initial percentage decline in active colonies occurred whether abundance was increasing or decreasing. The later percentage increase began only while numbers were decreasing.

As infestations aged, even active colonies decreased in size and activity, but the year of minimal density was accompanied by increases in colony size and vitality. This rejuvenation also was reflected in data from adults and egg masses. Methods of using egg-mass data to determine the proportions of different kinds of colonies in infestations of different ages are discussed, and adult activity indices based on scale loss and wing tearing are presented. Examples from all sources indicated declining vitality while the population aged, followed by sudden recovery at or near minimal abundance when its least viable portion had been eliminated.

Introduction

In 1956, an outbreak of the western tent caterpillar, *Malacosoma pluviale* (Dyar), reached its peak in the Saanich Peninsula of southern Vancouver Island, British Columbia. Within the outbreak, local infestations varied in age and intensity. A few were only in their first generation, and very light. Others, in their fourth year of residence, were much heavier. Noticeable differences in population quality accompanied these differences in age and abundance (6).

The qualitative differences were most striking in the larval stage, during which they were revealed by the appearance and habits of whole colonies. In newly infested localities, most colonies were very active. In older infestations, some were active, but many were very sluggish—often too much so to survive.

These differences were traceable to individuals that differed in their behavior, developmental rate, and survival ability from eclosion onward. Some were

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active and well orientated: others were not. The proportions of these active and sluggish individuals varied enough among colonies to produce the differences observed there and between infestations.

The preliminary observations of 1956 left several questions unanswered. There was no information on the causes of the persistent individual differences. Although they seemed bound to affect the animal's population dynamics, their ultimate importance was unknown, and it was not always clear how their existence could be exploited in population studies. For example, would they show annual as well as local changes during future variations in abundance? If so, would their changes have predictive value? Finally, there was the question why some areas had supported populations throughout the period of increasing abundance, while others were successfully invaded only during the year of maximum density.

Since 1956, populations in the outbreak area have been studied to obtain answers to these questions. This paper is the first of a series outlining events observed between 1956 and 1959, the period of population decrease. It deals with qualitative changes observed during this period that were common to all kinds of infestations. Consequently, it forms a necessary link between previous reports (6, 7) and future, more detailed contributions on factors influencing decrease and on distributional changes associated with changing abundance. Investigations of the causes of the differences among individuals were set aside after 1957 to await increases in abundance that would provide enough material for experiments.

The methods and content of this paper were limited by some peculiarities of the outbreak area that left the colonies which survived the outbreak collapse wholly accessible. This accessibility provided ideal conditions for field observations of changes in quality, abundance, and distribution, but it also had undesirable aspects. Fully accessible colonies were highly vulnerable to zealous collectors. Accordingly, to avoid disruption of natural trends by excessive collection, sample sizes and collecting areas were severely restricted while a system of surveys based almost entirely on direct observation was worked out. The content of the paper is based largely on results from the few localities where sampling seemed permissible during the interim. Occasionally, supporting information from the detailed observational surveys of the whole area has been included, but on the whole, local samples of small size have been used. Despite the sample sizes, evidence for the trends described is cumulative, and they are real enough. In any event, I preferred the sacrifice of a few significance tests in some of the following tables to the irrevocable loss of this whole population.

The Types of Individuals

Throughout the paper, it will be necessary to refer repeatedly to the types of individuals or their colonies. Accordingly, an outline of the initial differences and their biological consequences is inserted here. Readers are referred to previous publications (6, 8) for more details of the different kinds of behavior and their effects on development and survival.

The original method of separating the different types involved exposure of newly emerged larvae to light from a 30-w fluorescent tube. The larvae were spaced in rows on white paper so that they could not contact each other without travelling several times their own body length. Those that moved directly toward the lamp when isolated in this manner were separated from the others. The rest were brushed together into clusters and their actions during cluster breakup were observed. A few stragglers of the type mentioned above were detected during this interval, and then the remaining larvae were separated into additional categories according to differences in their orientation ability and total activity when they were reisolated.

In 1956, larvae of the first type were labelled type I, and all others were classed as type II larvae of differing abilities. Since 1957, however, three grades of type II larvae have been distinguished regularly. These are called types II*a*, *b*, and *c*, in decreasing order of activity. In reality, individual differences in activity form a continuously graded scale ranging from most active to moribund (or even to "unhatched eggs", when larvae are unable to emerge successfully). Orientation groups are somewhat more discrete. Nevertheless, four categories are more satisfactory than lengthier series when thousands of larvae must be handled quickly. And four categories are enough to reveal even small variations in the proportions of the different types among different colonies.

When they are exposed to light in the manner outlined above, type I larvae are capable of independent, directed movements while isolated from other members of their group. Consequently, on the paper they travel directly to the light. On twigs or on foliage, they are capable of invading new territory unmarked by the silk trails of other larvae. Since they are the most active individuals, they are the first to respond to any disturbing stimulus or to the return of suitable weather after conditions have been below thresholds for movement or feeding. Their movements stimulate some of the other types of individuals at such times, and in the resulting wave of activity that passes through the colony, most of its members follow the type I larvae to a different location. Consequently, their presence in appreciable numbers in a colony ensures that the general level of group activity will be high. Colonies that contain a high proportion of types I and II*a* larvae build several elongate tents during their first few instars, occupy two or more of these tents simultaneously, and forage over long distances.

Type II*a* larvae are relatively active, but they are incapable of independent orientation when isolated on flat surfaces, primarily because the amplitude of their major lateral searching movements is so great that they never proceed very far in a straight line. They can travel a straighter course in the presence of silk deposited by other larvae, or when placed alongside another larva so that the amplitude of their lateral body movements is damped. On large twigs where silk trails are absent, they cannot extend the range of colony activities very far unless literally pushed from behind by other larvae. Because their activity level is high, however, they are a valuable component of colonies in which the type I component may be small. Their presence in

appreciable numbers in such colonies helps to transmit activity waves through basking or resting groups, thus ensuring that the interval between feeding periods is not overly long.

Type II*b* larvae are so often completely undirected as individuals or as groups that they are really incapable of fending for themselves except in the immediate vicinity of their deposition point. There are occasional instances of a sort of "amoeboid flow" in pure groups of active II*b* larvae which enables them to proceed with painful slowness toward a light source, but any young caterpillar that had to depend on that sort of travel to and from its food supply would not be apt to grow much older. When they are small, therefore, they depend for their ultimate suvival on the more directed types of individuals or the silk trails these deposit, if their food supply is more than a few centimeters distant. As they grow older and larger, they are capable of more extensive forays without assistance, but they tend to stay relatively close to their sheltering tent in the absence of stimulation by other larvae.

Although they are not an especially active component of a colony, II*b* larvae form the major group in colonies that have very different levels of general activity. Hence, they comprise the bulk of the tent caterpillar population. In any colony, their presence favors the survival of a fraction of the original group for several reasons. One of their more important contributions is in tent construction, during which they deposit denser, tougher mats of silk than the more active larvae, primarily because they move about less, so that their silk strands are concentrated, not widely dispersed. A stronger, less permeable tent results, which guards the whole colony against excessive desiccation and predation. In addition, their numbers help the colony to absorb losses to predators or parasites that are sporadic in their attacks. (Persistent predators such as ants or earwigs generally decimate a young colony regardless of the proportions of different types it contains.)

Nevertheless, colonies that consist largely of II*b* larvae and that lack sufficient numbers of more active types are apt to experience slow starvation in the midst of plenty, because their low level of activity limits their foraging to foliage near the tent. Concentration of attacks on this foliage soon reduces both its quantity and quality, with resultant increases in larval mortality. Colonies of this sort rarely make more than two tents, and spend the greater part of their larval life enlarging one, which takes a compact form.

Type II*c* larvae are the weakest members of a colony. Some fail to emerge from their eggs, and many die soon after eclosion. Their numbers are reduced by further mortality throughout the larval stage, often near periods of ecdysis, so that few survive to maturity without special care in the laboratory or exceptionally favorable circumstances in the field. On emergence, they are very sluggish. They seldom do more than move their heads while isolated and, indeed, they move about very little even when they are in contact with other larvae. This sluggishness frequently results in decreased feeding opportunities for them in natural situations, because they often are left behind when the rest of the colony moves out to feed. In fact, many remain behind and starve to death when a colony abandons a tent and moves to a new site.

It is unlikely that any II*c* individual in a natural colony ever contributes to the next generation. Despite this failure, type II*c* is an important component of colonies, because its presence in too great numbers, when added to the II*b* component, produces a colony that is simply too sluggish to survive. If a colony has a large percentage of active individuals, its initial II*c* component generally is small and is further reduced by being left behind on abandoned tents. When there are few active individuals, however, the initial II*c* component is large by comparison and, since sluggish colonies rarely construct many tents, all living II*c* larvae are apt to remain with the rest of the colony. At the outset, they contain no more disease than any other category, but their habits promote the rapid spread of disease that may appear from any source, so that larval mortality within such colonies mounts rapidly. More often, they are detrimental only because their sluggishness contributes to the kind of self-inflicted starvation noted above, which kills many members of colonies so affected and depletes the vigor of any survivors.

The following tabulations show how these differences affect colonies in natural situations. While larvae were available in sufficient quantities for experimental purposes, it was customary to make up artificial colonies to determine the effects of different proportions of the four types of individuals on colonial life. Table I shows the proportions of the types in one group of colonies assembled during 1957, and Table II shows the effect of these proportions on colonial behavior and development in two different environments.

TABLE I

The numbers of first-instar larvae of *M. pluviale* of different types used to make up artificial colonies exhibiting different levels of activity, 1957

	Colony type	
	Active	Sluggish
No. larvae/colony		
Type I	44	6
Type II*a*	38	24
Type II*b*	92	110
Type II*c*	26	60
Totals	200	200

The numbers shown in Table I were calculated by determining the percentages of the various categories in typical natural colonies and rounding these to quantities that would allow equal totals to be used in the preparation of the four artificial colonies. The final composition of each colony, therefore, was closely comparable to examples encountered in natural situations. The colonies were prepared by mixing larvae from different egg masses to allow similar chances for the occurrence of latent virus disease. They were given comparable exposure to the sky 4 feet above ground on adjacent trees in each habitat listed in Table II.

TABLE II

Behavior and development of four artificial colonies placed on *Salix* sp. in two different habitats, 1957

		Colony type			
		Active		Sluggish	
Habitat		A[a]	B[b]	A	B
No. tents constructed during instar I		4	3	1	1
Total tents constructed		7	5	2	1
No. usually occupied simultaneously		2	2	1	1
Max. length of final tent (in.)		6	6.5	4.5	3.5
Shape of final tent[c]		E	E	C	C
Final length of tent construction area (ft)		4	2.5	0.6	0.3
Foraging distance (ft)		5	5	0.6	1
Cumulative no. leaf clusters damaged to end of instar	I	13	14	6	8
	III	32	55	13	20
No. days from eclosion to end of instar	I	15	15	20	20
	III	30	26	39[d]	36[d]

[a]Cool, moist, often shaded.
[b]Warm, moist, seldom shaded.
[c]E = elongate; C = compact.
[d]Killed by virus 2–3 days before the end of instar III.

In Table II, the figures for tent construction are graphic illustrations of the behavioral consequences of changing the proportions of the different categories per colony. The length of the tent construction area and the foraging distance are measurements of the lengths of twigs and smaller branches covered by the colony in its travels. They do not include the dimensions of leaf clusters attached to the twigs. The figures indicate, for example, that active colony "A" constructed its total of seven tents over a branch distance of 4 feet, and its larvae travelled an additional foot while feeding, to make a total foraging distance of 5 feet. Sluggish colony "A", on the other hand, constructed its two tents on less than 1 foot of branch, and never fed beyond the boundaries of the construction area.

The number of leaf clusters damaged by each colony and the number of days required to reach the end of the third instar differed markedly between colony types, but they also reflected environmental influences within each activity group. During the first instar, the two habitats received nearly equal amounts of insolation. As solar elevations increased later in the season, habitat "B" received more sunlight. The effects of this additional warmth are shown in the increased food consumption and developmental rates of the "B" colonies.

Mortality from various causes was recorded within each colony until all the members were dead or the survivors had entered their fourth instar. Table III shows the numbers of larvae per colony accounted for by the different mortality factors. Parasitism is not listed because it did not become prevalent in 1957 until the fourth instar. In the artificial colonies, only four

TABLE III

The distribution of mortality within the four artificial colonies, 1957

Habitat	Colony type			
	Active		Sluggish	
	A	B	A	B
Abandoned on vacated tents to die of starvation	15	20	19	0
Dying later of starvation or physiological weakness	3	2	20	39
Disappeared (dying alone after wandering from colony, or killed by predators while wandering)	75	56	20	28
Killed by spiders near tent	10	0	4	2
Killed by ants on tent or feeding highways	0	120	0	0
Killed by virus	40[b]	2	137[a]	131
Surviving to early fourth instar	57	0	0	0
Totals	200	200	200	200

[a,b] Include one and two parasitized third-instar larvae, respectively.

larvae were actually parasitized before then, and three of these were killed by virus a few days later.

Both active colonies abandoned most of their type II*c* larvae when they left their first tents. A number of II*c* larvae in sluggish colony "A" were abandoned in the same way, but its original complement was large enough to leave additional individuals to die later from starvation, physiological weakness, and disease.

Differences in disease mortality between the two kinds of colonies express differences in habit. Disease was present in all colonies. It was just starting to appear in active colony "B" when the larvae began to spin a tent on a branch that was a main highway for foraging ants. Within a day, they were all carried off by the ants. Deaths from disease in active colony "A" recurred during the third instar, but continuing tent construction and subdivision of the colony between two occupied tents kept down the infection rate. In contrast, continual contacts among clustering larvae weakened by starvation in the two sluggish colonies led to their ultimate destruction by virus just before they reached the end of their third instar, i.e., as they entered the period of stress just before ecdysis. These mass eliminations by disease support Chitty's contention (3) that it is more profitable to examine earlier events in an animal's life than those immediately associated with death when qualitative differences are involved. The previous habits, experience, and physiological weakness of members of the sluggish colonies were directly involved in their ultimate elimination by virus.

The greater numbers of wandering larvae lost from active colonies arose from the tendency of active individuals to lead small groups of followers into new territory. Not all active colonies lose so many wanderers in the early instars. In the present examples, however, climate was harsh and predators were plentiful on the open-grown trees. Ants and spiders accounted for many larvae lost at a distance from the main foraging areas. The remainder died

of exposure when they did not return to the colony, since no small larva can survive in natural situations without intermittent rests in its tent during its early instars. Deaths from these factors are grouped in the table because it was not possible to locate every individual that strayed.

Laboratory classification of the 57 survivors showed that there were 7 type I, 11 type II*a*, 36 II*b* (one of which was parasitized), and 3 II*c* larvae. Less than 16 and 29% survival occurred among I and II*a* larvae, respectively. Most of their losses occurred during wandering, but some were killed at the tent by spiders, which tend to trap the first (i.e., the most active) individuals to leave a tent during the morning feeding period. Thirty-nine per cent of the II*b* component survived, and the three surviving II*c* larvae amounted to 11% of the original number.

These tables have been discussed at some length because it is possible to reduce similar observations on natural colonies to statistics for comparative purposes. In fact, the items listed in Table II are now recorded each year for all colonies encountered during detailed surveys. They are especially valuable for comparative surveys during low densities because they can be obtained by direct observation without disturbing a colony. Consequently, they will be met again in this and later contributions.

Changes in Natural Populations

Numerical Changes in the Outbreak Area

In the autumn of 1955, a roadside survey of abandoned tents established the boundaries of the infestations for subsequent annual comparisons. Some 154 miles of roadways were utilized, but this mileage was later extended to 156.4 to provide a more adequate survey grid. The grid provides almost total coverage of the undisturbed parts of the area, because tents there tend to occur on roadside vegetation instead of in the wood lots behind. This tendency has provided unusual opportunities for obtaining annual records of absolute numbers during the period of collapse and subsequent decrease.

Table IV summarizes population changes from 1956 to 1959, based on surveys during the fourth larval instar. The 1956 figures are estimated from a series of spot samples, and may contain errors amounting to 1000 colonies. Colony figures for subsequent years are total counts, and the only other estimate involved is in the 1957 larval data, where the error may amount to 1000 larvae. The remaining larval counts are correct.

TABLE IV

The numbers of *M. pluviale* along 156 miles of road at the time of the annual fourth-instar survey

	1956	1957	1958	1959
No. colonies	≈74,000	3,164	390	251
No. larvae	≈6,000,000	≈193,000	16,182	16,975
Average no. larvae/colony	81	61	41	68

The decrease from 1956 to 1958 shows why sampling collections were restricted to avoid disrupting natural trends in numbers and quality. The additional decrease in 1959 colony numbers is real, but the increases in the total number of larvae and the average number per colony show that the decline in abundance is ending. Data discussed later show, in fact, that an upsurge in quality is associated with these recent numerical increases. Although the improvement is still restricted to only a few localities, it is marked enough there to affect the figures for the over-all trend.

Changes Foreshadowed by Egg-mass Data

Some of the most valuable information on qualitative changes may be obtained from records of larval types emerging from egg masses, if eggs are sufficiently numerous to permit collection. Unrestricted collections were not advisable in 1958–59, but general information is included in Table V to show 1956–59 changes in the whole population comparable with the numerical changes in Table IV. The next few tables show how 1957 records were utilized in connection with population age structure.

In Table V, significance tests were not advisable because the 1958–59 quantities were so small in relation to the size of the collecting area. Nevertheless, the trends illustrated agree with those indicated by other lines of evidence, and can be accepted as real. Before 1959, the decline in abundance shown in Table IV was accompanied by decreases in the size of egg masses and in the numbers of their emerging larvae (Table V). There was also a steady decrease in the number of type I larvae per mass associated with an increasing percentage of type II*c* larvae per egg mass when the latter were grouped with their less successful companions, the eggs which failed to hatch (paragraph 3, the types of individuals). All percentages were based on the number of eggs per mass available for hatching, because some egg masses had a few eggs parasitized or chewed by arthropod predators, which invalidated comparisons based on total number of eggs per mass.

TABLE V

Annual comparisons of *M. pluviale* egg masses field-collected in February and hatched in March and April

	1956	1957	1958	1959
$\bar{x}$ total eggs	216.34±11.58	200.82±6.29	155.14±12.63	170.25±7.78
$\bar{x}$ emergence	200.81±11.31	174.98±6.41	115.43±11.63	160.94±8.42
$\bar{x}$ type I larvae	27.00± 2.17	20.86±2.08	14.43± 2.20	21.66±2.05
$\bar{x}$ type II*c* larvae	31.42± 3.48	36.68±3.37	20.00± 3.55	23.50±3.16
$\bar{x}$ unhatched eggs	10.22± 2.33	10.21±2.47	24.00± 3.01	6.94±1.48
%[a] type I larvae	12.79	11.23	10.34	12.90
%[a] type II*c* larvae	14.89	19.81	14.34	14.00
%[a] type II*c* larvae + unhatched eggs	19.73	25.32	31.56	18.13
n (egg masses)	32	56	17	16

[a]Percentages of eggs available for hatching, i.e., those not chewed or parasitized.

The increased viability in some areas indicated in 1959 (Table IV) also is evident in the egg-mass records of Table V. Despite such signs of recovery, 1959 colonies hatched from these eggs were returned to the natural population, as were those of 1958, to avoid interference with naturally occurring trends. In contrast, colonies from 1957 egg masses were kept separate for further testing in connection with work shown in the next few tables.

The 1957 egg masses were collected from areas known to differ in their previous infestation history. When the eggs were divided accordingly, the proportions of the different types of emerging larvae showed variations that confirmed 1956 observations of colonial differences between young and old infestations (6). Table VI shows the significant differences in numbers of active and sluggish larvae per colony that existed at eclosion among populations which had occupied areas for different periods. The numbers of types I and II*a* larvae per egg mass decreased as infestation age increased, whereas the number of II*c* larvae varied directly with infestation age. As a result, emerging colonies in the youngest 1957 infestation had roughly one active larva for every 1.8 sluggish larvae they contained (Table VI: 65 I+II*a* larvae vs. 119 II*b*+II*c* larvae per colony). This infestation was beginning its second resident generation in 1957. In contrast, infestations that were already heavy in 1955 were at least 5 years old by 1957, and their ratios of active to sluggish larvae per colony approximated 1:4.4. Moreover, in young infestation colonies, the 65 active larvae were associated with only 27 II*c* larvae, whereas in old infestation colonies, the 30 active individuals had approximately 45 II*c*'s obstructing colony activity and development. As indicated previously, the number of II*b* larvae per egg mass did not differ significantly among colony types destined for very different activity levels.

It is possible to predict the type of tent a colony should form as soon as its emerging larvae have been classified. Individual colonies that should form elongate tents during subsequent development generally contain more than 13% type I, more than 29% I+II*a* larvae, and fewer than 21% type II*c* larvae at the time of eclosion. Colonies sluggish enough to form compact

TABLE VI

Mean numbers of the different types of larvae emerging in three infestations of different ages, 1957

	Infestation history		
	Absent in 1955	Light in 1955	Medium to heavy in 1955
No. eggs/mass	205.11±13.29	211.37±10.34	186.21± 9.55
Emergence/mass	183.72±12.28	177.89±11.70	163.79±10.32
Type I larvae/mass	34.95± 3.34[a,b]	17.00± 2.99[a]	11.37± 2.25[b]
Type II*a* larvae/mass	30.00± 3.10[a,b]	22.16± 2.17[a]	18.89± 2.26[b]
Type II*b* larvae/mass	91.44± 5.57	101.47± 6.91	88.58± 7.17
Type IIc larvae/mass	27.33± 4.44[a]	37.26± 5.57	44.95± 7.09[a]
n (egg masses)	18	19	19

[a,b] $P < 0.05$ for these mean differences between infestations.

tents have fewer I's and II*a*'s, and more than 21% type II*c* larvae. Sluggish colonies may be subdivided further into those that are potentially viable in favorable environments, and those that are moribund, i.e., incapable of surviving past their first instar. The latter have fewer than 3% type I, fewer than 13% I+II*a*, and more than 30% II*c* larvae. Therefore, an egg-mass sample from any infestation can be classified according to the percentages of each of these types of colonies it contains as soon as the larvae have emerged. In 1957, this was done with the egg masses used in Table VI, and the results are shown in Table VII.

Table VII shows the egg-mass data arranged according to the criteria given above. The number of moribund sluggish colonies per infestation (C) increased markedly with infestation age. The number of active colonies, on the other hand, decreased as infestation age increased (A).

The percentages shown in Table VII are those which existed at eclosion. The ratio of active:sluggish colonies per infestation is subject to an abrupt change soon after development begins whenever there are moribund sluggish colonies in the population. Later in the season, more gradual changes in

TABLE VII

The egg masses of Table VI arranged to show the variations in activity and viability to be expected from their colonies, 1957

	Infestation history		
	Absent in 1955	Light in 1955	Medium to heavy in 1955
No. egg masses yielding			
A. Active colonies with elongate tents	11 (61.1%)	8 (42.1%)	5 (26.3%)
B. Sluggish colonies (compact tents) capable of surviving to the fourth instar in a favorable environment	7 (38.9%)	7 (36.8%)	6 (31.6%)
C. Moribund sluggish colonies incapable of much development past instar I	0 (0.0%)	4 (21.1%)	8 (42.1%)
n	18	19	19

NOTE: A = Colonies with more than 13% type I larvae, more than 29% I+II*a* larvae, and fewer than 21% type II^c larvae after eclosion.
B = Colonies with fewer than 13% type I, fewer than 29% I+II*a*, and more than 21% II^c larvae.
C = Colonies with fewer than 3% type I, fewer than 13% I+II*a*, and(or) more than 30% II^c larvae.

the ratio occur as potentially viable sluggish colonies are eliminated one by one. These colonies are consistently more vulnerable to weather and to certain types of predators, such as wasps, than active colonies are. Therefore, the ratio changes as development proceeds, and it may be very different at the time of a fourth-instar survey than it was at eclosion. Consequently, the test colonies were reared outdoors in 1957 to observe these changing proportions, and also to provide a basis for comparison among the actual infestations during the later fourth-instar survey.

Survey information on quality is most conveniently expressed in terms of the percentage of active colonies per infestation. The top line of Table VIII, therefore, repeats the eclosion percentages of active colonies shown in the

TABLE VIII

Changes in the percentages of active colonies per sample during subsequent larval development in the colonies of Table VII, 1957

	Infestation history		
	Absent in 1955	Light in 1955	Medium to heavy in 1955
% active colonies at eclosion (from Table VII)	61.1	42.1	26.3
% at the end of instar I, after the moribund sluggish colonies had died	61.1	53.3	45.5
% in the early third instar, after the loss of additional sluggish colonies	64.7	57.1	55.6
% change between emergence and early third instar	+3.6	+15.0	+29.3

preceding table. Abrupt changes in these percentages occurred in the samples from older infestations as the moribund sluggish colonies were eliminated during the first instar. Thereafter, more gradual changes occurred as other sluggish colonies died. By the time observations were terminated in the early third instar, no active colony had died, but losses of sluggish colonies had changed the percentages to the values shown. The net changes per infestation are listed at the bottom of the table.

Although these samples were small in terms of absolute numbers, they were relatively large in comparison with actual 1957 populations in the infestations from which they came. Comparison of the third-instar percentages of active colonies in Table VIII with the 1957 percentages shown in Table IX shows that properly classified samples give surprisingly accurate indications of what may be expected to occur in natural situations. The differences between third-instar percentages in the test populations and fourth-instar percentages in the infestations were only 1.0, 4.8, and 2.6% in the young, intermediate, and old infestations, respectively. In these infestations, no empty elongate tent was found during 1957 surveys, but empty compact tents were evident. Although this does not preclude the possibility of some mortality among active colonies at a very early stage, it does indicate that the pattern of natural mortality was very similar to that observed in the test colonies.

Changes in Aging Populations

The egg masses discussed above were collected along 1-mile strips of road in the three different infestations. Table IX shows the annual changes in abundance and quality that have occurred in these localities since 1956. In this and similar tables, quality is expressed by showing the percentages of active colonies that form elongate tents. This method stems from 1956 observations that new infestations suddenly appearing in areas far removed from other foci consisted exclusively of active colonies during their first generation. Differences between these and older infestations in 1956 led to the assumption that later resident generations would have decreasing percentages of active colonies as a new infestation aged.

TABLE IX

Annual comparisons of colonies at the beginning of the fourth instar in the three infestations listed in earlier tables[a]

	Infestation history					
	Absent in 1955		Light in 1955		Medium to heavy in 1955	
	Total colonies	% with elongate tents	Total colonies	% with elongate tents	Total colonies	% with elongate tents
1956[b]	<30	>80	≈300	>70	≈800	45
1957[c]	108	65.7	63	61.9	67	58.2
1958[c]	2	50.0	10	50.0	6	66.7
1959[c]	1	100.0	5	100.0	5	80.0

[a]One mile of roadside surveyed in each infestation.
[b]Estimated values.
[c]Total counts.

Table IX shows that the pattern of events was not quite so simple. From 1956 to 1958, there were annual decreases in the percentages of active colonies in the youngest and in the intermediate infestations. But in 1959 the percentages rose again because only active colonies existed by the time of the fourth-instar survey. In the oldest infestation, there was a steady rise in the percentages of active colonies from 1956 to 1959. Because the numbers for the later years in Table IX were so small, I have included Table X to show that the changes in these short distances were not unique. Increasing the survey distance in the two extreme infestations changed some of the actual percentages, but left the trends unchanged.

To understand what occurred, it is necessary to consider the impact of trends common to the whole outbreak upon localized infestations within it. In 1956, the year of peak population, the oldest infestation was in its fourth generation. Its previous period of increase, therefore, closely paralleled the period of general increase for the whole outbreak prior to 1956. Similarly, its subsequent decline in abundance paralleled the general decline. In contrast, the youngest infestation was established in the year of peak population, and its first increase coincided with the year of general collapse. Thereafter, its population declined with the rest of the outbreak, but at an accelerated rate.

What occurred in the youngest infestation after 1956 was simply a more compressed version of what occurred in older infestations both before and after 1956. Shifting the time-scale in Tables IX and X so that the year 1958 for the youngest infestation corresponded to the year 1956 for the oldest infestation clarified the pattern of events. During the first three or four resident generations, the percentage of active fourth-instar colonies in either area declined to levels near 50%. Thereafter, the trend reversed, and annual increases in the percentage of active colonies occurred. When densities were lowest, percentages of active colonies tended to reach 100% where only short distances were considered, because no sluggish colony occurred. A few sluggish

TABLE X

Annual comparisons of colonies at the beginning of the fourth instar in additional areas where infestations were absent in 1955 and where they were medium to heavy in 1955. (Compare with Table IX)

	Infestation history			
	Absent in 1955		Medium to heavy in 1955	
	Total colonies	% with elongate tents	Total colonies	% with elongate tents
1957	996	66.2	753	59.5
1958	100	55.0	102	67.6
1959	51	88.2	89	79.8
Mileage	35.7		18.9	

colonies could be found when greater distances were examined, however, so that percentage increases in active colonies did not necessarily reach such high values.

The original hypothesis that decreasing quality arising from increasing periods of residence in an area should be expressed by reduced numbers of active colonies (6) needs some modification, therefore, to relate it to actual trends in the outbreak. In fact, the idea that only isolated new infestations consist solely of active colonies also needs some revision. The changes that must be made are discussed later, but they involve the appearance and content of colonies in different kinds of infestations. Accordingly, the next section presents survey information on the annual changes within colonies from different infestations.

Changes within Colonies

Tables XI and XII contain the same type of information presented earlier in Table II, but now it is derived from natural colonies observed in young and old infestations. Since the tables span the period, 1957–59, and the same areas were surveyed each year, the terms "young" and "old" require qualification. They are convenient terms derived from the 1956 status of each infestation, and cause no difficulty so long as one remembers that a "young" infestation of 1956 was 4 years old in 1959.

Table XI shows annual changes in maximum tent length, number of tents, foraging distance, and number of larvae observed among active colonies in the two kinds of infestations. Because this information was obtained during the fourth-instar survey, some data on parasitism and disease could be included. Table XII presents the same information for sluggish colonies, so that comparisons should be made between as well as within the tables.

Discussion here is restricted to some obvious and consistent trends because of several difficulties with records from restricted localities. For example, significance tests are applicable to most parts of the tables, but comparisons of this sort are hampered by the small number of sluggish colonies available in the sampling localities in 1959. Moreover, preliminary observations made

TABLE XI

Annual comparisons of active colonies at the beginning of the fourth instar in young and old infestations

Infestation status, 1957	1957		1958		1959	
	Young	Old	Young	Old	Young	Old
$\bar{x}$ tent length, in.	5.98±0.41	5.67±0.25	5.93±0.35	5.61±0.69	5.56±0.39	5.70±0.36
$\bar{x}$ no. tents/colony	2.60±0.26	2.26±0.26	1.21±0.13	1.77±0.22	1.78±0.19	2.15±0.18
$\bar{x}$ foraging distance (ft)	3.16±0.23	3.13±0.29	3.06±0.43	2.35±0.21	4.64±0.63	4.43±0.45
$\bar{x}$ no. larvae/colony	80.60±5.53	64.96±3.39	63.61±6.44	52.24±5.25	54.37±6.51	60.75±3.21
% colonies attacked by dipterous parasites	33.3	51.9	42.1	22.2	5.6	0
% colonies with diseased larvae	0	0	0	0	16.7	5.0
n (colonies)	30[a]	27[a]	19[b]	27[b]	18[b]	20[b]

[a]Samples approximating 15% of total colonies in young areas, 19% in old areas.
[b]Total counts in both areas. Mileages surveyed in 1957–58: 6.4 in young areas, 5.7 in old areas; in 1959: 11.0 in young areas, 9.4 in old areas.

TABLE XII

Annual comparisons of sluggish colonies at the beginning of the fourth instar in young and old infestations

Infestation status, 1957	1957		1958		1959	
	Young	Old	Young	Old	Young	Old
$\bar{x}$ tent length, in.	3.46±0.30	3.44±0.12	3.24±0.19	3.30±0.33	—	3.29±0.37
$\bar{x}$ no. tents/colony	1.89±0.27	1.61±0.17	1.55±0.19	1.27±0.12	—	1.71±0.35
$\bar{x}$ foraging distance (ft)	1.19±0.16	1.22±0.13	1.21±0.16	1.16±0.22	—	1.48±0.32
$\bar{x}$ no. larvae/colony	53.06±5.89	44.06±3.27	41.82±4.25	38.62±3.38	—	47.86±6.44
% colonies attacked by dipterous parasites	22.2	5.6	22.2	46.7	—	14.3
% colonies with diseased larvae	5.6	16.7	0	0	—	0
n (colonies)	18[a]	18[a]	18[b]	15[b]	0[b]	7[b]

[a,b]See Table XI.

while techniques were still being developed during 1956 cannot be compared directly with later observations. This is unfortunate, because the most striking changes in sizes of tents, feeding areas, and colonies occurred between 1956 and 1957.

Despite the lack of tabular comparisons for 1956, the reader may gain some idea of colony sizes then by examining the tent photographs in the first publication of this series (Figs. 63–71; 6). In 1956, elongate tents larger than 7 inches were common, and many were longer than 10 in. Larval numbers per active colony were generally more than 100 at the fourth instar, and foraging distances in such colonies averaged well over 5 feet. Similarly, compact tents with sides longer than 4 inches were common, and their larval numbers ranged above 65–70. Their feeding areas, however, were not very different from those shown for 1959 sluggish colonies in Table XII. Until 1959, no colony of either kind comparable with those of the peak year was observed anywhere in the outbreak area. Even in 1959, there were only a few as large as average 1956 colonies.

In 1957, both types of colonies decreased in size to the averages shown in the tables, and this trend persisted through 1958 in both infestations. The 1958 colonies, though often not differing significantly in their average values

from those of 1957, were very different in appearance. Many of their larvae were stunted, and their tent silk was weaker and tore more easily than in previous years. As a result, even active colonies appeared somewhat slovenly in 1958. By 1959, some colonies showed great improvements in size and appearance. Tent silk was tougher, though tents were still small. Larvae were larger. In fact, after colony breakup, many mature larvae reached lengths near 5 centimeters. They approximated 1956 sizes and were a full centimeter longer than in 1958.

In addition, many of the differences between colonies in young and old infestations were finally reversed in 1959. Earlier, the colonies in young infestations were consistently larger. This was especially true of active colonies (Table XI). In 1959, active colonies in the young infestations increased their numbers of tents per colony and their foraging distances over 1958 values, but their tent sizes and larval numbers continued to decrease. In contrast, 1959 colonies in the "old" infestations were larger than in previous years. They also appeared to be in better condition than colonies in the young infestations.

Some of these differences were not so apparent in sluggish colonies (Table XII), in which foraging distances, for example, approached minimal values in most years. One difference occurred in 1959, however, when the young infestations sampled contained no sluggish colony capable of surviving to the fourth instar, with equivalent colonies in young infestations outside the sampling localities faring little better. In contrast, old infestations sampled yielded seven sluggish colonies, and there were additional colonies in outside areas. All showed enough improvement to warrant the contention that surviving sluggish colonies in "old" infestations of 1959 were better than those of 1958.

Comparison of Tables XI and XII shows that sluggish colonies were consistently and significantly much smaller than their more active counterparts, with an important exception. In areas or years of minimal vitality, larval survival in active colonies differed little from that in the larger sluggish colonies of better years. (Compare active colonies, "old" 1958 and "young" 1959 with sluggish colonies, "young" 1957.) This was another aspect of the continued deterioration in the quality of the population as the outbreak subsided.

The effects of colony type on successful parasitism are still not fully understood. There is no doubt that individual larvae or colonies too sluggish to reach maturity even in favorable circumstances sometimes act as parasite traps that waste larval parasites deposited upon them. This definitely occurred among the old sluggish colonies in 1958, because none of their larvae survived long enough to allow parasites to mature. In fact, most parasites deposited on members of any sluggish colony during periods of population decrease undoubtedly are wasted because of this inability of their hosts to survive long enough. Whether this operational error by parasite adults severely limits their efficiency, however, I am not yet prepared to say, because it may be counterbalanced to some extent by the fact that active colonies in most

localities often are the first to reach the age most vulnerable to parasites. The quantitative importance of parasite wastage, therefore, depends upon annual differences in synchronization of host and parasite. The annual differences shown in the tables accordingly reflect local density differences in parasite populations less than they reflect annual imperfections in the synchronization of host development with adult parasite flight period. When active colony development was properly aligned with the peak of parasite oviposition, active colonies suffered most. When they developed quickly enough to reach colony breakup before the oviposition peak, however, their larvae escaped many attacks. Then, sluggish colonies were most heavily parasitized. More seasons of observation are required, however, before the population consequences of these changes in the type of colony attacked can be worked out.

The records for virus disease are more straightforward. Disease was widespread in 1956. It was still present in 1957, though it occurred most frequently in sluggish colonies in the earlier stages of larval development. In the sampling areas, it was restricted to sluggish colonies (Tables XI and XII). In 1958, it was no longer present in the sampling areas. In fact, only four colonies in the whole outbreak area showed any signs of it during the fourth-instar survey, and three of these were active colonies. By 1959, this interesting reversal in the type of colony attacked was complete. In that year, the only colonies that contained diseased larvae were active ones forming elongate tents (Table XI). This was true in all parts of the outbreak, and it raises a point concerning virus transmission between generations during periods of low density.

Spontaneous appearance of virus and bacterial diseases in tent caterpillar colonies is established (1), though ovarian transmission may still be a controversial subject. I have indicated previously that sluggish colonies are not apt to produce any fertile adults during their period of lowest vitality, which coincides with the last years of population decrease. If virus is to be transmitted by adult females, therefore, it must be carried by fertile members of active, not sluggish colonies during this period of reduced abundance. The fact that active colonies were the only ones exhibiting diseased larvae in the period of lowest density strongly suggests that this is what actually occurred.

The comparative weakness of sluggish colonies continues to exact its toll as long as colony unity is maintained. In fact, traces of its effects can be seen even after colony breakup has become general. Thus, the proportions of the different colonies per infestation are subject to further changes before the population reaches the pupal stage, especially when densities are higher. Two examples from 1957 records, the last year in which the phenomena occurred, will make this clear.

When colonies are more numerous, as in 1957 (Table X), disease is especially noticeable just before fifth-instar larvae begin to leave their colonies for the period of solitary feeding that precedes prepupational travel. Further losses of whole colonies occur during this interval but, at these higher population densities, sluggish colonies are more affected. Thus, the numbers of sluggish colonies in the young and old infestations of Table X were reduced by 22.7

TABLE XIII

The numbers of different types of tents still occupied after fifth-instar dispersal was generally complete along 24 miles of road, 1957

	Degree of exposure to the sky			
	Fully shaded		Fully exposed	
	No. larvae remaining			
	<20	>40	<20	>40
Elongate tents	0	5	4	2
Compact tents	0	0	7	12

and 25.0%, respectively. In the same infestations, the numbers of active colonies were reduced by 17.2 and 20.5%, respectively.

Also at higher densities, the end of the period of fifth-instar dispersal leaves some tents still occupied. Most of these are occupied by colonies retarded by their own weaknesses, but a few may be located in unfavorable environments that delayed development. Table XIII shows how these colonies could be classified during 1957. Its records were obtained by repeating the tent survey along 24 miles of road after dispersal seemed complete. In this distance, there were 861 abandoned tents and 30 still occupied. The latter were distributed as shown in the table.

Those with fewer than 20 larvae contained only sluggish stragglers which would not complete development. These were only the residue of a colony otherwise safely dispersed. Four elongate tents in fully exposed sites were in this category, which meant that their stragglers had managed to remain alive until colony breakup. In the absence of stimulation by more active members of their colonies, they were not foraging, but were dying slowly of starvation on their tents. Seven fully exposed compact tents also fell in this category. There was no tent of either type with larval numbers between 20 and the next category.

Tents with more than 40 larvae were divisible into three kinds. In the first, in very shaded situations, a few active colonies had managed to survive the lack of sunshine, though their development was so delayed that they were just entering their fourth instar. The larvae were healthy, however, and since they had escaped parasitism by being too small earlier in the season, they were maturing unharmed. There was a notable lack of compact tents in this "shaded" subdivision. No sluggish colony of 1957 was capable of surviving in such an environment.

The second subdivision consisted of fully exposed elongate tents that still harbored most of their larvae. These were sluggish and flaccid, generally heavily parasitized, but obviously dying of starvation more rapidly than from parasitism. Growth of leaves along the feeding highways indicated that there had been a sudden reduction in the foraging distance during the preceding weeks. These colonies, of which only two examples were found, had obviously lost their entire active component suddenly, and the remaining members were unable to fend for themselves.

The third subdivision, similar to the one above, consisted entirely of compact tents harboring sluggish colonies. There were 12 of these compared with only 2 of the elongate tents. As noted, this final evidence of weakness among sluggish colonies is most noticeable at higher densities. As populations decline further, weaker colonies generally succumb long before fifth-instar dispersal becomes general.

Changes in Adult Activity

Each year since 1956, some colonies were collected just before they dispersed, and the larvae they contained were reared to the adult stage. Adults were classified according to differences in their total activity. From 1957 to 1959, the larvae also were classified in the four categories previously outlined. Tables XIV and XV show the annual variations in adult activity observed (see also pp. 305, 310 in ref. 6).

The adult activity indices in the tables were obtained by allowing the moths to emerge without disturbance in 5×8 cm jars in the laboratory. Each was left undisturbed in its jar for a further 2 days, and then examined to determine the amount of scale loss and wing tearing it exhibited. Individual activity indices were allotted according to the following code:

1. Perfect or nearly so. (Perhaps a few scales missing from one wing edge.)
2. Minimal fraying of forewing edges, not accompanied by loss of scales elsewhere or of thoracic pile.
3. Moderate fraying of wing edges, minor scale loss, and minor wear of thoracic pile.
4. Extensive fraying of wings, moderate scale loss, and total wear of thoracic pile.
5. Bad tearing of wings, often reducing them to stubs. Well-nigh complete scale loss on remaining parts.

Higher index values denote increased activity. Groups of individuals could be used to obtain mean values of the indices for special purposes, and the annual changes in these means are shown in the tables. In Table XIV, 1956 records are not included because the field-collected mature larvae were not

TABLE XIV

Annual comparisons of activity indices of field-collected adults previously classified by larval type at the end of the fourth instar

	Larval type				
	I	II*a*	II*b*	II*c*	*n*
$\bar{x}$ male indices					
1957	4.14	3.16	2.75	2.03	87
1958	3.67	2.43	2.00	2.00	42
1959	4.30	3.59	3.38	3.20	73
$\bar{x}$ female indices					
1957	3.00	2.53	2.00	1.53	63
1958	3.33	2.83	2.30	2.00	25
1959	3.45	3.05	2.72	2.20	92

TABLE XV

Annual comparisons of activity indices of field-collected adults from young and old infestations

	Infestation history			
	Absent in 1955		Medium to heavy in 1955	
	Index	n	Index	n
$\bar{x}$ male indices				
1956	3.82±0.07	33	2.94±0.14	53
1957	3.10±0.23	29	2.69±0.15	58
1958	2.80±0.27	15	2.11±0.14	27
1959	3.80±0.16	45	3.46±0.19	28
$\bar{x}$ female indices				
1956	3.36±0.16	11	2.10±0.37	10
1957	2.29±0.19	24	2.05±0.12	39
1958	2.79±0.27	14	2.45±0.26	11
1959	2.72±0.15	36	2.91±0.11	56

classified by type in that year. Also, the 1958 categories were too small to have meaningful standard errors, so none is included in the table. Consequently, Table XIV shows only the differences that may be expected to occur, though it may be used in conjunction with Table XV. The 1958 increase in the female indices of Table XIV, for example, appears again in Table XV. It is certainly real, since its population consequences have been illustrated in several of the preceding tables.

Annual changes in the index values of the sort shown in both tables arise through shifts of the population up or down the activity scale. Thus, in declining populations, vigor decreases result in the production of a smaller proportion of the most active adults, and this lowers the index value. When populations become more vigorous after their weakest component is eliminated, larger proportions of the more active individuals occur, and the index increases. Consequently, the index is a valuable and sensitive tool for annual survey use, even when it is derived from small samples. It may be used, for example, to assess fertility in local infestations to determine whether they are maintaining their own populations or are being supported by immigrants. Such applications of the index are treated more fully in later contributions.

The male indices in both tables decreased steadily until 1959, then suddenly increased. A comparable earlier increase in female activity occurred in 1958 in all types of individuals in both kinds of infestations. This resurgence among females was essential to the recovery observed in all stages of the 1959 generation, and necessarily preceded it. The continued increase in female activity in the "old" infestations during 1959 was another aspect of the general recovery already noted in those areas.

Discussion

Quality resides in the individual, but its population consequences cannot be ignored. Previous authors (2, 4, 5, 6) have commented on the probable

consequences of changes in the environmental requirements or the intrinsic viability of animals from one generation to the next. Each considered different aspects of the problem, but all had one point in common: population ecologists are unwise to assume that the properties of individuals are constant within or between generations.

A recent paper by Chitty (3) provides further evidence for the importance of individual quality and includes a restatement of the pertinent hypothesis: ". . .all species are capable of limiting their own population densities without either destroying the food resources to which they are adapted, or depending upon enemies or climatic accidents to prevent them from doing so . . . under appropriate circumstances, indefinite increase in population density is prevented through a deterioration in the quality of the population." Chitty then points out that reduced vitality of this sort would make it possible for independent events, such as weather, to have increasingly severe effects upon a population in which quality fell as numbers rose.

These views provide opportunities for linking ostensibly conflicting theories in a way that would bring welcome relief to readers of population literature. They are worth careful assessment, therefore, even by those who are satisfied with one or another of the major theories now in circulation. Chitty himself points out (3) that his hypothesis, "can be falsified. . . by proving that there are no significant differences between expanding, stationary, and declining populations in the distribution of the properties of the individuals." On these terms, the present results support the hypothesis, because they all show consistent qualitative differences among individuals or groups from different kinds of populations. Furthermore, the nature and extent of these differences make them an adequate mechanism for affecting population density "under appropriate circumstances". Any discussion of the effects of qualitative differences on tent caterpillar population dynamics, however, must take into account the modifying influence of colonial life upon individual habits and survival.

When populations consist of free-living individuals that develop separately, the quality of each individual before its reproductive age may be largely its own concern. Similarly, its death before maturity may be a private affair that need not affect any other individual nearby. In contrast, both the qualities and deaths of immature individuals have immediate communal aspects in tent caterpillar colonies that make these groups an important factor in the animal's population dynamics.

A colony is more than a unit that must produce a pair of adults to maintain a given population density. A "good" colony, provided it can survive at all, not only protects its best members but also insulates its II*b* members from their own ineptitude and from environmental stresses they could not survive in the absence of better-orientated individuals. In exceptionally favorable environments, therefore, colonial life promotes the survival of weaker, as well as stronger individuals. When its surviving members mature, the strongest fliers among them *may* oviposit locally, but they also are capable of flying farther away beforehand: and many do. In contrast, the less active adults

are incapable of sustained flight, so that all females of this type that are able to oviposit must do so near their birthplace. Thus, partial emigration of the better stock coupled with complete retention of poorer quality, but as yet still viable stock hastens the decline of population quality in the locality (6). In this way, colony life leads to the deterioration of local population quality most rapidly when it is functioning most effectively, i.e., during periods of maximum survival. So long as the environment permits survival of the poorer and poorer colonies produced by an increasingly sluggish resident population, their numbers increase locally to the point where they far outnumber any better colonies produced by the few active adults which oviposit locally or which immigrate from outside. Ultimately, the presence of numerous colonies too sluggish to reproduce or even to survive under the most favorable circumstances affects population density in the locality. When they die, there is an abrupt decrease in local abundance of the sort postulated by Chitty (3).

Moreover, in fluctuating environments where the climate changes suddenly, the annually increasing sluggishness of resident populations also leads to sudden reductions in numbers. Colonies too weak or disorientated to survive the first season of bad weather are suddenly eliminated. In addition, intermediate colonies also are lost to the population, because when their weaker members succumb, the remaining members are not sufficiently numerous to provide the silk and mass of insulating bodies required for survival in the more rigorous environment. Once again, therefore, there is an abrupt decrease in abundance.

The population consequences of the events outlined above appear in the tables of this paper. Because of these important aspects of colonial life, discussion of larval qualities, especially, is confined more to colony units than to individuals. The impact of different individuals on colony life is clear enough. The observations on artificial colonies (Tables II and III) showed some of the consequences of behavior and activity differences during development in different environments. In difficult environments, for example, the exceptional activity of several individuals led to increased losses or even to total loss of the colony. In any environment, however, too much sluggishness led inevitably to total loss.

Extension of the measurements of colony activity to natural colonies (Tables XI and XII) showed persistent differences in size and activity between active and sluggish colonies. There was also evidence of a gradual averaging of the population during its decline in numbers by elimination of the least viable colonies at one extreme and further deterioration of the quality of the best colonies at the other. This qualitative deterioration continued into 1958 and, since there was no food shortage after 1956, nor excessive parasitism or disease after 1957, it was consistent with the argument that intrinsic weaknesses in the population are as important as any other biotic factor affecting population decline.

Some aspects of deteriorating quality during these years were more noticeable than measurable. Changes in larval size were mentioned earlier. In

addition, the durability of tents showed great annual differences, from a maximum in 1955 to a minimum in 1958. While the population was still expanding, tents were so durable that more than 50% of those vacated by fifth-instar larvae in June, 1955, remained recognizable until mid-May, 1956. Each year thereafter, durability decreased until 1958, when no tent persisted more than a month after it was vacated. Abandoned tents are severely affected by rain, but the differences observed were not simply the result of seasonal weather differences. The summer of 1958, for example, was notable for its dry weather after the tents were abandoned, but they still deteriorated quickly.

There were also differences in adult behavior most easily observed between generations of females. In 1956, several unmated females in the emergence jars deposited small clumps of eggs and patches of spumaline before they died, as lepidopterous adults often do in unnatural situations. This did not occur again until 1959, when it once more became common, but only among the more active females.

Such noticeable differences in behavior and appearance help to distinguish new from very old infestations when both contain only elongate tents. In 1956 it was shown that new infestations isolated from other foci by more than 0.5 mi at first contained only elongate tents. This seemed a valuable tool for surveys until it was noticed in 1958 that very old infestations with densities of $\leqslant 2$ colonies per mile also contained only elongate tents where the colonies were spaced at intervals of 0.5 mi or more. These colonies, however, had small, battered tents, and their larvae were few and stunted in comparison with those in isolated new infestations. The original rule that only new infestations contain elongate tents can still be used, therefore, with the additional provision that these tents must be large, supporting large colonies of healthy larvae.

Tables IX and X showed how initially high percentages of elongate tents in a locality first declined with increasing years of residence, then began to increase again. The initial decline actually was mainly associated with increasing term of residence, since it occurred independently of local increases or decreases in abundance. It was direct evidence, therefore, of the deteriorating quality of local populations previously discussed. Even better evidence of this qualitative decline was provided by increases in the proportion of nonviable sluggish colonies as local infestation age increased (Table VII), but this evidence was more elusive, and had to be sought through examinations of egg-mass data. The youngest infestation of Table VII, for example, had no moribund sluggish colonies in 1957, but the intermediate infestation produced 4/19, or 21%, and the oldest had 8/19, or 42%.

These increases in viable and moribund sluggish colonies were behind the ultimate increase in the proportion of active colonies in the locality. The annually increasing number of moribund colonies led to larger increases in the percentages of active colonies early in larval life (Table VIII), and increasing losses of potentially viable sluggish colonies occurred as the environment became less favorable. In many parts of this outbreak, these events went

hand in hand, resulting in sudden, then continuing annual increases in the proportion of active colonies per locality.

In the year their proportion began to increase again, many of the active colonies were the small, slovenly type previously mentioned. Further improvement in population quality occurred after weaker colonies of this sort died or failed to produce any adult capable of ovipositing. Afterwards, only the better colonies of the population were concerned with the next generation, and qualitative improvements of the sort shown in 1959 occurred (Table XV, 1958 females; Tables XI and XII, 1959 colony statistics).

Changes in the environment, especially in the climate, were intimately linked with these changes, but there is not space to provide sufficiently detailed demonstrations of the manner in which the other factors operated. I have tried here to outline the type of biological substrate on which they worked, while indicating also that sluggish colonies were consistently more susceptible to cool, shady, moist environments (Tables II and XIII). For future reference, however, it is worth noting that sometimes the weather became so bad that no colony could survive in some localities. Then, the resulting distributional changes were associated with rearrangements of quality and abundance throughout the rest of the outbreak area.

Tables derived from different life history stages and from diverse methods of observation or collection have shown that consistently decreasing vitality characterized the period, 1956–58, and that signs of recovery were most common in 1959. There is other evidence (6) that the last part of the period of expansion (just before 1956) also was characterized by more localized declines in vitality wherever resident populations had been established for more than one generation. Consequently, the pattern of events observed thus far has been consistent with Chitty's conclusions concerning vole populations. The reasons for declining vitality, however, may prove somewhat different for tent caterpillars than for voles.

Conclusions

1. Larvae emerging from *M. pluviale* egg masses are easily separable into four categories on the basis of their orientation efficiency and total activity. Type I larvae are active and orientate well when isolated from other members of their group. No other type can orientate while isolated, but type II*a* larvae are very active and can orientate in groups. Type II*b* larvae are less active and remain disorientated individually or in groups. Type II*c* larvae are very sluggish and seldom complete development.

2. Colonies with more than 13% type I larvae, more than 29% type I+II*a* larvae, and fewer than 21% II*c* larvae are active and construct several elongate tents during their first three instars. Those with fewer I's and II*a*'s, but more II*c*'s, are less active, and generally construct only one or two compact tents in the same period. Those with fewer than 3% type I, fewer than 13% I+II*a*, and (or) more than 30% II*c* larvae are moribund, and usually die before the end of their first instar.

3. Comparatively young infestations only 1 or 2 years old contain no moribund colonies, and generally contain a high proportion of active colonies, especially when they are isolated from other infested areas. The number of egg masses producing moribund colonies increases with infestation age, as does the proportion of living sluggish colonies that produce compact tents. These differences are detectable at eclosion, and persist through the larval stage, although initial proportions of the different viable colonies are modified by environmental action before the larvae enter their fourth instar.

4. When egg masses are available, it is possible to calculate the proportions of the different colonies to be expected later in the different infestations simply by classifying the emerging larvae and sorting the colonies into categories indicated above. The difference between the proportions of these colonies determined at emergence and the final proportions observed in the field during the fourth instar gives some measure of differential environmental mortality during the interval.

5. Close observation of natural colonies or of artificial colonies made up to resemble natural units reveals the ways in which this environmental mortality occurs in different kinds of colonies in various habitats. More active colonies suffer many of their losses during wandering of small groups led by well-orientated larvae. The most sluggish colonies are eliminated simply because they do not wander enough, but stay on their tents to starve or fall prey to disease.

6. From 1956 to 1959, the number of fourth-instar colonies in the outbreak area was reduced from approximately 74,000 to 251. In 1959, however, the number of larvae per colony increased over values for the 2 previous years, and the total number of larvae in the outbreak area increased slightly from the 1958 low of 16,182 to 16,975.

7. Many indicators of qualitative change within the population were associated with the numerical changes. Total eggs and total emergence declined prior to 1959, then increased in that year. Similar changes occurred in the numbers of the more active individuals among the annual crops of emerging larvae. In the field, the proportion of active fourth-instar colonies per infestation fell to levels near 50%, then began to rise again. Colony sizes and measurements which expressed the level of colony activity also decreased until 1959, when 1956 values were regained by a few colonies, and all showed some improvement over 1958. These improvements were most noticeable in parts of the oldest infestations. Finally, the activity indices of adult males and females, based on the amounts of self-inflicted wing damage displayed by emerging adults, fell in all populations until 1958, when female indices began to rise again.

8. In each locality, the initial decrease in the proportion of active colonies was an early result of the deteriorating quality of the resident stock. The later increase in the proportion of active colonies per locality resulted from the elimination of the less viable and the moribund sluggish colonies from the population by extrinsic and intrinsic mortality factors. The first decrease

in the proportion of active colonies occurred whether population density was rising or falling. The ultimate increase occurred only after a period of decreasing density.

9. Results lend support to Chitty's hypothesis that populations are capable of limiting their own densities through deteriorations in quality that occur whether or not extrinsic mortality factors are operating, and that such reductions in vitality may lead to increasingly severe effects of some mortality factors, such as weather, upon the weakened population.

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References

1. BUCHER, G. E. Winter rearing of tent caterpillars, *Malacosoma* spp. (Lepidoptera:Lasiocampidae). Can. Entomologist, **91**, 411–416 (1959).
2. CHITTY, D. Adverse effects of population density upon the viability of later generations. *In* The numbers of man and animals. Oliver and Boyd, Ltd., Edinburgh. 1955. pp. 57–67.
3. CHITTY, D. Population processes in the vole and their relevance to general theory. Can. J. Zool. **38**, 99–113 (1960).
4. FRANZ, J. Über die genetischen Grundlagen des Zusammenbruchs einer Massenvermehrung aus inneren Ursachen. Z. angew. Entomol. **31**, 228–260 (1949).
5. KENNEDY, J. S. Phase transformation in locust biology. Biol. Revs. Cambridge Phil. Soc. **31**, 349–370 (1956).
6. WELLINGTON, W. G. Individual differences as a factor in population dynamics: the development of a problem. Can. J. Zool. **35**, 293–323 (1957).
7. WELLINGTON, W. G. Meteorology in population dynamics. Intern. J. Bioclimatol. Biometeorol. 2 (Pt. III, Sect. B) (1958).
8. WELLINGTON, W. G. Individual differences in larvae and egg masses of the western tent caterpillar. Can. Dept. Agr. Forest Biol. Div. Bi-monthly Prog. Rept. **15** (6) (1959).

NEMATODE PARASITES OF VERTEBRATES OF EAST PAKISTAN

IV. ASCAROID NEMATODES FROM AMPHIBIA, BIRDS, AND MAMMALS[1]

S. P. GUPTA[2]

Abstract

Three new species, *Porrocaecum* (*P.*) *haliasturi* sp. nov., *Aplectana agubernaculum* sp. nov., and *Aplectana asiatica* sp. nov., are described from Dacca, East Pakistan, and four species, *Contracaecum* (*C.*) *haliaëti* (Baylis and Daubney, 1923), *Heterakis spumosa* (Schneider, 1866), *Heterakis beramporia* (Lane, 1914), and *Meteterakis govindi* (Karve, 1930) Inglis, 1957, redescribed. *Toxocara mystax* (Zeder, 1800) and *Heterakis gallinae* (Gmelin, 1790) Freeborn, 1923 are recorded from the domestic cat and fowl.

Heterocheilidae Railliet and Henry, 1915

Porrocaecum (*Porrocaecum*) *haliasturi* sp. nov. (Figs. 1–6)

Host: Kite (*Haliastur indus*).

Location: Small intestine.

Material: Two females and one male.

Description

The worms are large, stout, and coiled. The cuticle is transversely striated. The mouth (Fig. 5) is surrounded by three lips, a dorsal one bearing two large papillae and two subventral, each bearing two small papillae. The pulp of the dorsal lip bears two flattened, expanded processes. The interlabia, as seen from the outer surface, are triangular with broad bases. The dentigerous ridges are extremely fine. The oesophagus is more or less uniformly thick and possesses a short, oblong ventriculus (Fig. 6). There are three valves at the end of the oesophagus which project into the intestine. The intestinal caecum (Fig. 6) is well developed and extends along the oesophagus for a considerable length. Caudal alae are absent. The excretory pore is situated close to the nerve ring. The vulva is a little in front of mid-body.

Male.—75 mm long, 1.11 mm wide. Head rounded (Fig. 1), 0.31 mm in diameter. Dorsal lip 0.19 by 0.24 mm. Interlabia (Fig. 2) 0.14 to 0.16 mm long. Nerve ring 0.78 mm, excretory pore 1.09 mm from anterior end. Oesophagus 4.44 mm, ventriculus 0.67 mm, and intestinal caecum 2.77 mm in length. Tail

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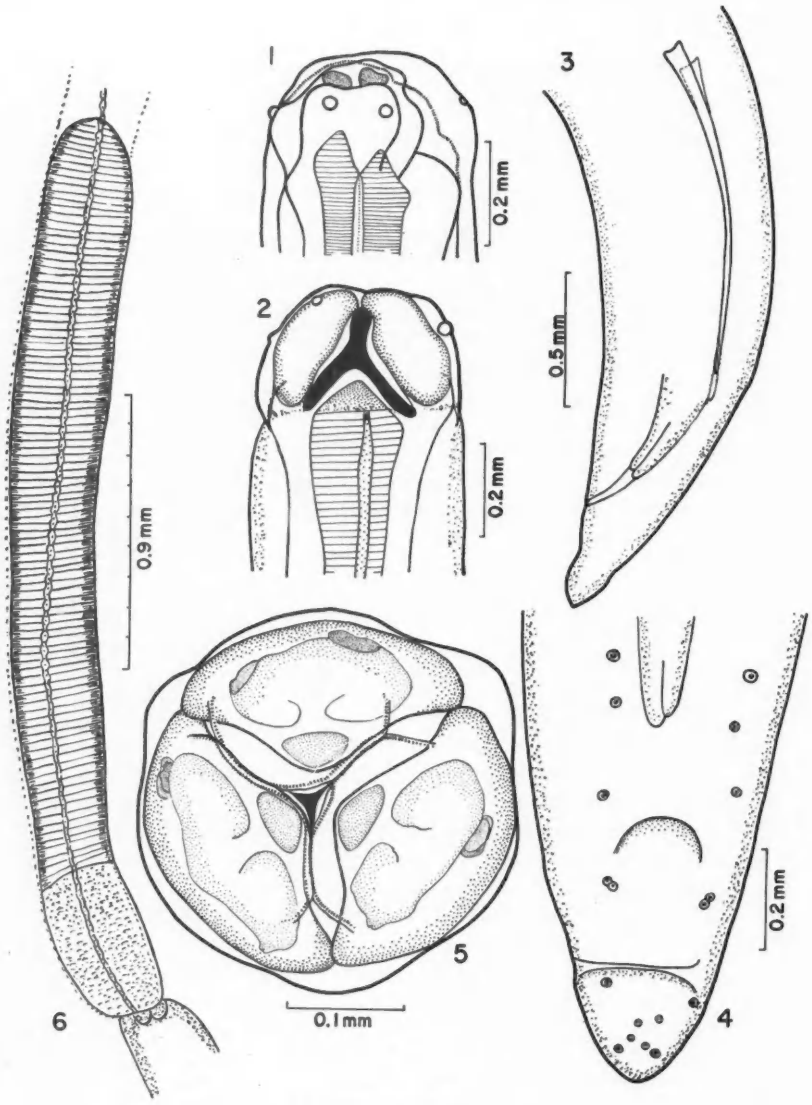
[2]National Research Council of Canada, Postdoctorate Fellow.



FIGS. 1–6. *Porrocaecum* (*P.*) *haliasturi* sp. nov.
FIG. 1. Head of male, dorsal view. FIG. 2. Head of male, dorsolateral view. FIG. 3. Male tail, lateral view. FIG. 4. Male tail, ventral view. FIG. 5. End-on view. FIG. 6. Junction of oesophagus and intestine showing intestinal caecum.

(Fig. 3) conical, slightly curved ventrally, 0.36 mm long, constricted 0.22 mm from tip. Twenty-two pairs preanal and five pairs postanal papillae. First postanal papillae double (Fig. 4), situated immediately behind cloaca; others behind constriction arranged in tandem by pairs; two large, in same subventral row as preanal papillae; two small, ventral in position. Hindmost preanal papillae situated close to cloaca. Spicules (Fig. 3) similar and subequal, right 1.28 mm, left 1.3 mm long. No gubernaculum.

Female.—95 to 110 mm long, 1.18 to 1.3 mm wide. Head 0.31 to 0.34 mm in diameter. Dorsal lip 0.22 to 0.23 mm by 0.16 to 0.19 mm. Interlabia 0.12 to 0.17 mm long. Nerve ring and excretory pore 0.84 to 0.95 mm and 0.97 to 1.07 mm, respectively, from anterior end. Oesophagus 4.87 to 4.91 mm, ventriculus 0.48 to 0.52 mm, and intestinal caecum 2.91 to 3.13 mm in length. Tip of tail blunt. Tail 0.55 to 0.73 mm long, with constriction behind anus. Vulva slightly anterior to mid-body 45 to 53 mm from anterior end. Eggs thin-shelled, subglobular, 65 to 80μ by 55 to 70μ.

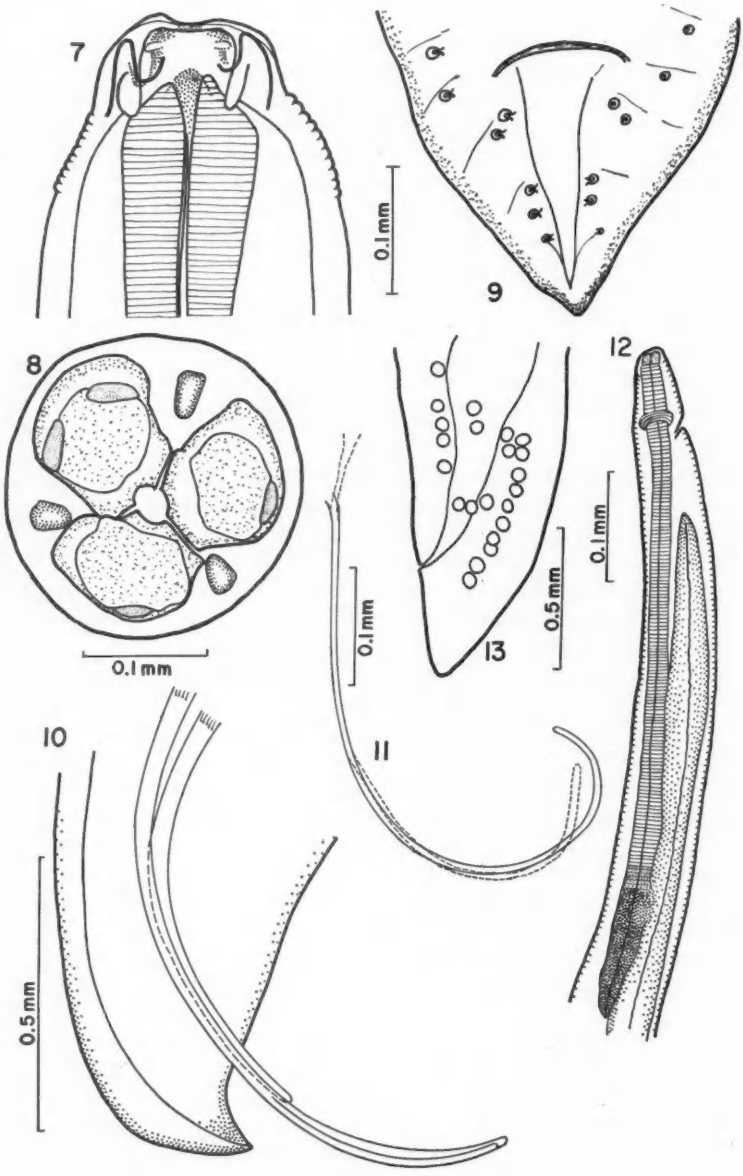
Discussion

Porrocaecum has not hitherto been recorded from *Haliastur indus.*

Skrjabin (20) recently divided the genus *Porrocaecum* into two subgenera, *Porrocaecum* and *Laymanicaecum.* The subgenus *Porrocaecum* is characterized by the absence of an accessory piece, the subgenus *Laymanicaecum* by the presence of one. Due to the absence of an accessory piece the new form comes under the subgenus *Porrocaecum.*

Seven species, *Porrocaecum* (*Porrocaecum*) *crassum* (4), *P.* (*P.*) *depressum* (5), *P.* (*P.*) *angusticolle* (5), *P.* (*P.*) *ardae* (4), *P.* (*L.*) *reticulatum* (4), *P.* (*P.*) *cheni* (8), and *P.* (*P.*) *wui* (8), have been described from Asiatic birds. The present species differs from *P.* (*P.*) *cheni*, and *P.* (*P.*) *wui* (Hsü, 1933) from China, in having large, well-developed intestinal caeca, in the number and arrangement of preanal and postanal papillae, and in the structure of the eggs which in the new form are not elliptical in shape and the shell of which is neither pentagonally reticulated nor surrounded by a corrugated coat. It differs from *P.* (*L.*) *reticulatum* in the absence of an accessory piece, from *P.* (*P.*) *ardae* in the absence of lateral alae, from *P.* (*P.*) *crassum* in having the vulva anterior to mid-body and in not having alate spicules, and from *P.* (*P.*) *depressum* in the character of the anterior lobes of the lip in a pulp, in the number of preanal papillae, and in the structure of the eggs which are not thickened at the poles.

The new form resembles *P.* (*P.*) *angusticolle* (5) in the shape of the lobes of the pulp of the dorsal lip, and in the arrangement of the postanal papillae, but differs in (*a*) the absence of a longitudinal ridge or fold of cuticle on either side towards the base of the lip in both sexes; (*b*) the number of preanal papillae, (*c*) having subequal spicules about one-third longer, (*d*) the position of the vulva (which lies a little anterior to mid-body), and (*e*) the absence of caudal papillae on the female tail. It also resembles *P.* (*P.*) *phalacrocoracis* Yamaguti, 1941 (27) in the number and arrangement of preanal and postanal papillae and in the shape of the spicules, but differs from it in the structure of the lips



FIGS. 7–13. *Contracaecum* (*C.*) *haliaëti* (Baylis and Daubney, 1923).
FIG. 7. Anterior region of male, dorsal view. FIG. 8. End-on view. FIG. 9. Male tail, ventral view. FIG. 10. Male tail, lateral view. FIG. 11. Spicules. FIG. 12. Anterior region of female, lateral view. FIG. 13. Female tail, lateral view.

and eggs, in the less anterior position of the vulva, and in the absence of a pair of caudal papillae on the female tail. Accordingly, this species is regarded as new and is named *Porrocaecum* (*Porrocaecum*) *haliasturi* sp. nov.

Contracaecum (*Contracaecum*) *haliaëti* (Baylis and Daubney, 1923) (Figs. 7–13)

Host: Kite (*Haliastur indus*).

Location: Small intestine.

Material: Eight females, eight males.

Contracaecum (*C.*) *haliaëti* (6) belongs to the subgenus *Contracaecum* due to the absence of a gubernaculum and a spoon-shaped depression on the lip. As this species has not hitherto been described adequately and as no complete description of a male is available, it is redescribed.

Description

The worms are large and stout. The cuticle is thick, with transverse striations. The annulations (Fig. 7) behind the lips are very deep. The mouth is surrounded by three lips each with two papillae and two anterior projections. Interlabia (Fig. 8) are present and conical. The oesophagus (Fig. 12) is club-shaped with a small ventriculus and an appendix. The intestinal caecum narrows anteriorly. The excretory pore is close to the nerve ring. The vulva is slightly in front of mid-body.

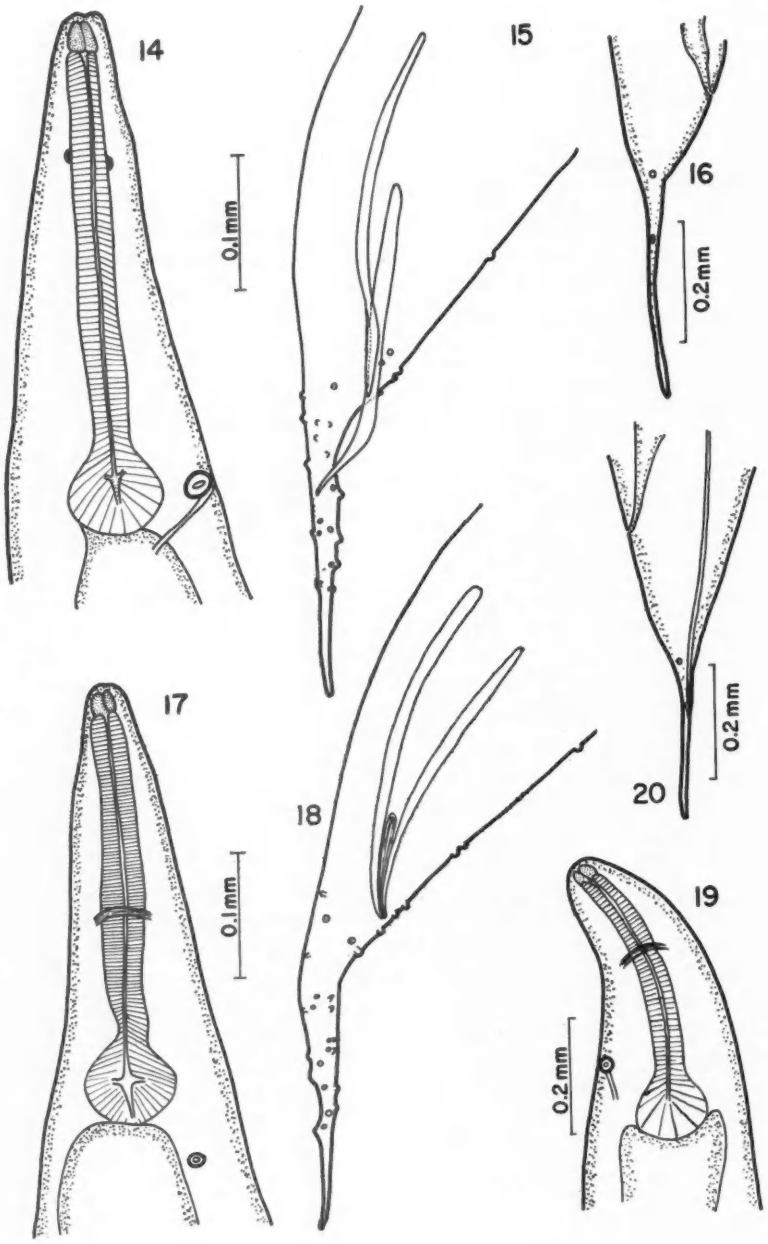
Male.—26 to 39 mm long, 0.81 to 1.1 mm wide. Diameter of head at base 0.13 to 0.17 mm. Cervical papillae 0.62 to 0.78 mm from anterior end. Dorsal lip 0.09 to 0.1 mm by 0.07 to 0.09 mm. Oesophagus including ventriculus 4.1 to 5.2 mm long; posterior appendix 0.69 to 1.18 mm long. Nerve ring 0.55 to 0.74 mm and excretory pore 0.59 to 0.78 mm from anterior end. Tail (Fig. 10) short, curved ventrally, 0.19 to 0.26 mm. Five pairs postanal papillae (Fig. 9), 1 adanal, and 34 preanal, arranged in regular series. Spicules subequal (Fig. 11), of relatively great length, consisting of a tubular shaft and short anterior expansion. Right spicule 5.9 to 6.7 mm long, left 6.5 to 6.9 mm. No gubernaculum.

Female.—39 to 46 mm long, 1.07 to 1.55 mm wide. Diameter of head at base, 0.12 to 0.24 mm. Cervical papillae 0.62 to 0.73 mm from anterior end. Dorsal lip 0.08 to 0.1 mm by 0.08 to 0.1 mm. Oesophagus including ventriculus 9.04 to 11.6 mm and posterior appendix 0.78 to 1.3 mm in length. Nerve ring 0.6 to 0.73 mm and excretory pore 0.66 to 0.78 mm from anterior extremity. Tail (Fig. 13) short, pointed, 0.31 to 0.34 mm long, slightly constricted behind anus. Vulva situated in front of mid-body 16 to 19.1 mm from anterior end. Eggs thin-shelled, spherical, 0.05 to 0.06 mm by 0.06 to 0.07 mm in size.

Ascaridae Cobbold, 1864

Toxocara mystax (Zeder, 1800)

A large number of male and female specimens were recovered from the small intestine of a domestic cat from Dacca, East Pakistan.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Cosmocercidae Travassos, 1925

Aplectana agubernaculum sp. nov. (Figs. 14–16)

Host: Rana tigrina.
Location: Small intestine.
Material: Numerous females and one male.

Description

These worms are small and thin, tapering at each extremity. The mouth has three lips each with two papillae. The pharynx is short. Narrow lateral alae extend throughout the body length. The excretory pore is a little anterior to the hind region of the oesophageal bulb. The tail tapers to a fine point in both sexes but that of the male curves sharply ventrally. There is no gubernaculum. The vulva is about mid-body.

Male.—1.86 mm long, 0.26 mm wide. Pharynx 0.024 mm long. Oesophagus including bulb 0.37 mm long; bulb 0.08 mm in diameter. Nerve ring 0.09 mm from anterior end. Excretory pore in oesophageal region (Fig. 14) 0.36 mm from anterior end, prominent, 0.02 mm in diameter. Tail (Fig. 15) 0.24 mm long tapering to point. Nine pairs preanal, 1 pair adanal, and 21 pairs postanal papillae. Spicules subequal, tubular, measuring 0.25 to 0.27 mm.

Female.—2.96 to 5.54 mm long, 0.25 to 0.4 mm wide. Pharynx 0.03 mm long. Oesophagus including bulb 0.39 to 0.54 mm long. Bulb subglobular 0.08 to 0.12 mm by 0.1 to 0.14 mm. Nerve ring 0.15 to 0.18 mm and excretory pore 0.36 to 0.53 mm from anterior end of body. Tail (Fig. 16) 0.35 to 0.55 mm long, narrowing rapidly behind anus and ending in tapering filament; two pairs caudal papillae. Vulva at about mid-body, 1.5 to 2.8 mm from anterior end. Uterus distended with embryos and larvae.

Discussion

Attempts have been made by Railliet and Henry (18), Baylis and Daubney (7), Karve (12), Baylis (2, 3), Travassos (21), Walton (24, 25), and Ballesteros Marquez (1) to distinguish the species of the genera *Aplectana* and *Oxysomatium* but their distinguishing characters are so variable that the status of the species is very doubtful.

Ballesteros Marquez (1) considered the presence or absence of a gubernaculum, the relative size of spicules, and the prodelphous or amphidelphous disposition of uteri to be valid generic characters for the Cosmocercidae. On this basis he revised the family creating *Neyraplectana* for those species of *Aplectana* with prodelphous females and males with two subequal spicules and no gubernaculum, and *Neoxysomatium* for those of *Oxysomatium* with amphidelphous females and males with two equal spicules and gubernaculum, and

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FIGS. 14–16. *Aplectana agubernaculum* sp. nov.
FIG. 14. Anterior region of male, lateral view. FIG. 15. Male tail, subventral view.
FIG. 16. Female tail, lateral view.
FIGS. 17–20. *Aplectana asiatica* sp. nov.
FIG. 17. Anterior region of male, subventral view. FIG. 18. Male tail, subventral view.
FIG. 19. Anterior end of female, lateral view. FIG. 20. Female tail, lateral view.

Neoraillietnema for amphidelphous *Raillietnema* species with eggs arranged linearly *in utero* and males with two spicules and no gubernaculum. Accordingly, *Neyraplectana* comprises *N. crucifera*, *N. vellardi*, *N. schneideri*, *N. pintoi*, and *N. linstowi*; *Neoxysomatium* comprises *N. brevicaudatum* and *O. contortum*; and *Neoraillietnema* comprises *N. preputialis*.

Jorge da Silva (11) on the basis of the classification proposed by Ballesteros Marquez (1) has transferred *Aplectana chilensis* Lent and Freitas, 1948 and *N. meridionalis* Lent and Freitas, 1948 from *Aplectana* to the genus *Neyraplectana* Ballesteros Marquez, 1945.

The author does not agree with the views of Ballesteros Marquez (1) and Jorge da Silva (11) in transferring the species of *Aplectana* to the genus *Neyraplectana* as none of these characters is of more than specific value. There seem to be no important differences between the genus *Aplectana* and the genus *Oxysomatium* because in both, the position of the vulva from a little anterior to posterior to mid-body, the position of the excretory pore from a little anterior to the oesophageal bulb to just behind it, the relative size of the spicules, the number and arrangement of caudal papillae, the presence or absence of a chitinized valve in the oesophageal bulb, the prodelphous or amphidelphous disposition of the uteri in the female, and the internal or external hatching of the embryos are very variable characters. Therefore, it is a matter of convenience to consider *Aplectana* distinct from *Oxysomatium* rather than because of its taxonomic importance.

In the present communication the author follows the classification proposed by Travassos (21).

Aplectana agubernaculum sp. nov. differs from all the known species of the genus except *A. crucifer* Travassos, 1925 (21), *A. pintoi* Travassos, 1925 (21), *A. vellardi* Travassos, 1926 (21), *A. linstowi* York and Maplestone, 1926 (21), *A. preputialis* (Skrjabin, 1916) Travassos, 1931 (21), *A. schneideri* Travassos, 1931 (21), *A. chilensis* Lent and Freitas, 1948 (16), and *A. meridionalis* Lent and Freitas, 1948 (16), in the absence of an accessory piece.

Aplectana agubernaculum closely resembles *A. crucifer* in having the excretory pore in the oesophageal region and *A. pintoi* and *A. preputialis* in having a male of smaller size but differs, however, from these and other species in the number and arrangement of the caudal papillae, in having the vulva at mid-body instead of pre-equatorial (as in *A. chilensis* and *A. meridionalis*) and post-equatorial in *A. linstowi*. It can further be distinguished from *A. schneideri*, *A. pintoi*, and *A. meridionalis* in having spicules of larger size. It also differs from *A. crucifer*, *A. pintoi*, *A. vellardi*, and *A. chilensis* in having a smaller tail.

Aplectana agubernaculum differs from *A. macintoshii* (Stewart, 1914) Travassos, 1931 (12) from India in the absence of an accessory piece and in the number and arrangement of the caudal papillae. In *A. agubernaculum* there are 9 pairs of preanal, 1 pair of adanal, and 21 pairs of postanal papillae while in *A. macintoshii* there are 9 pairs of preanal and 18 pairs of postanal papillae.

Aplectana asiatica sp. nov. (Figs. 17–20)

Host: Rana tigrina and *Bufo melanostictus.*
Location: Small intestine.
Material: Numerous females and two males.

Description

These are small, thin worms which taper at each end. The mouth has three lips each bearing two papillae. There is a very short pharynx. Narrow lateral alae extend throughout the body length. The excretory pore is a little posterior to the hind region of the oesophageal bulb. In both sexes the tail tapers to a fine point but that of the male curves sharply ventrally. A well-developed crescentic accessory piece is present. The vulva is situated in the anterior half of the body.

Male.—1.91 to 2.66 mm long, 0.22 to 0.23 mm wide. Pharynx 0.02 to 0.03 mm long. Oesophagus, including bulb, 0.34 to 0.36 mm long; bulb 0.06 mm long, 0.07 mm wide. Nerve ring 0.18 to 0.2 mm from anterior end. Well-developed excretory pore (Fig. 17) behind oesophageal bulb 0.37 to 0.39 mm from anterior end. Tail (Fig. 18) 0.24 to 0.26 mm long. Seven pairs preanal, 1 pair adanal, and 23 pairs postanal papillae of which 10 are subventral, 9 subdorsal, and 4 in the median region. Spicules subequal and tubular and measure 0.26 to 0.32 mm and 0.29 to 0.38 mm respectively. Accessory piece 0.07 to 0.09 mm long.

Female.—3.43 to 6.34 mm long, 0.29 to 0.4 mm wide. Pharynx 0.03 to 0.04 mm wide. Oesophagus, including bulb, 0.44 to 0.51 mm long; bulb 0.1 to 0.13 mm long, 0.11 to 0.16 mm wide. Nerve ring situated 0.15 to 0.26 mm from anterior end. Excretory pore (Fig. 19) anterior to oesophageal bulb, 0.35 to 0.49 mm from anterior end. Tail (Fig. 20) 0.33 to 0.52 mm long, narrowing rapidly behind anus and ending in a tapering filament bearing two pairs papillae. Vulva in anterior half of body 1.36 to 2.9 mm from anterior end.

Eggs contain well-defined larvae some of which have apparently hatched *in utero.*

Discussion

Aplectana asiatica sp. nov. differs from all the known species of the genus with a gubernaculum except *A. acuminata* (Schrank, 1788) Railliet and Henry, 1916 (21), *A. punctatum* Walton, 1932 (syn. *Oxysomatium punctatum* Walton, 1932 (23)), and *A. lopsei* Jorge da Silva, 1954 (11) in having the vulva in the anterior region of the body. It differs from *A. acuminata* and *A. lopsei* in the number and arrangement of caudal papillae and also from *A. lopsei* in having a larger male tail and a smaller female tail. It resembles *A. acuminata* (Schrank, 1788) Railliet and Henry, 1916, *A. americana* Walton, 1929 (22), *A. longicaudata* Walton, 1929 (22), *A. ranae* (Walton, 1931) Ballesteros Marquez, 1945 (1), *A. brumpti* Travassos, 1931 (21), *A. baylisi* (Walton, 1933) (23) Lopez Neyra, 1944, *A. mexicana* Walton, 1940 (24), and *A. lopsei* Jorge da Silva, 1954 in having the excretory pore behind the oesophageal region but differs from these in the number and arrangement of the caudal papillae, in the larger size of the spicules. It differs from all these species except *A. acuminata*

and *A. lopsei* in the possession of a larger tail in both sexes and from *A. longicaudata*, *A. ranae*, *A. brumpti*, and *A. lopsei* in having a larger gubernaculum.

Aplectana asiatica closely resembles *A. macintoshii* (Stewart, 1914) Travassos, 1931 (12) from India but can be distinguished from it in the number and arrangement of caudal papillae. In *A. asiatica* there are 7 pairs of preanal, 1 pair of adanal, and 23 pairs of postanal papillae whereas in *A. macintoshii* there are 9 pairs of preanal and 18 pairs of postanal papillae. It differs from *A. macintoshii* in the possession of subequal spicules of larger size. In *A. asiatica* the spicules measure 0.26 to 0.32 mm and 0.29 to 0.39 mm long, respectively, while in *A. macintoshii* they are 0.24 mm long. It differs from *A. macintoshii* in having a gubernaculum of large size, 0.07 to 0.09 mm instead of 0.02 to 0.03 mm long, and in the position of the excretory pore which lies behind the oesophageal region in the male. It can also be distinguished from *A. macintoshii* in the position of the vulva which lies in the anterior half instead of the mid-region of the body.

A. asiatica can be distinguished from *A. agubernaculum* in the possession of a gubernaculum, in the number and arrangement of anal papillae, in having larger spicules, in the position of the excretory pore which lies behind the oesophageal region in the male, and in the position of the vulva, which lies in the anterior half of the body.

Heterakidae Railliet and Henry, 1914

Heterakis spumosa (Schneider, 1866) (Figs. 21–23)

Host: Domestic cat.

Location: Small intestine.

Material: One female and six males.

All species of the genus *Heterakis* except *H. spumosa* occur in birds. All previous reports of *H. spumosa* have been from rats whereas the present record is from a cat. It is possible, however, that the cat in this instance was parasitized only transiently after ingestion of infected rats.

Description

These worms are of medium size, slightly curved ventrally towards the anterior end. The male tail bears 10 papillae of which 2 are suctorial, 5 cloacal, and 3 postanal. Spicules are short, tubular, and equal. The vulva is anterior to mid-body. The oesophagus (Fig. 21) is narrow with a small anterior pharynx and a distinct pear-shaped bulb which is muscular and valvulated. The nerve

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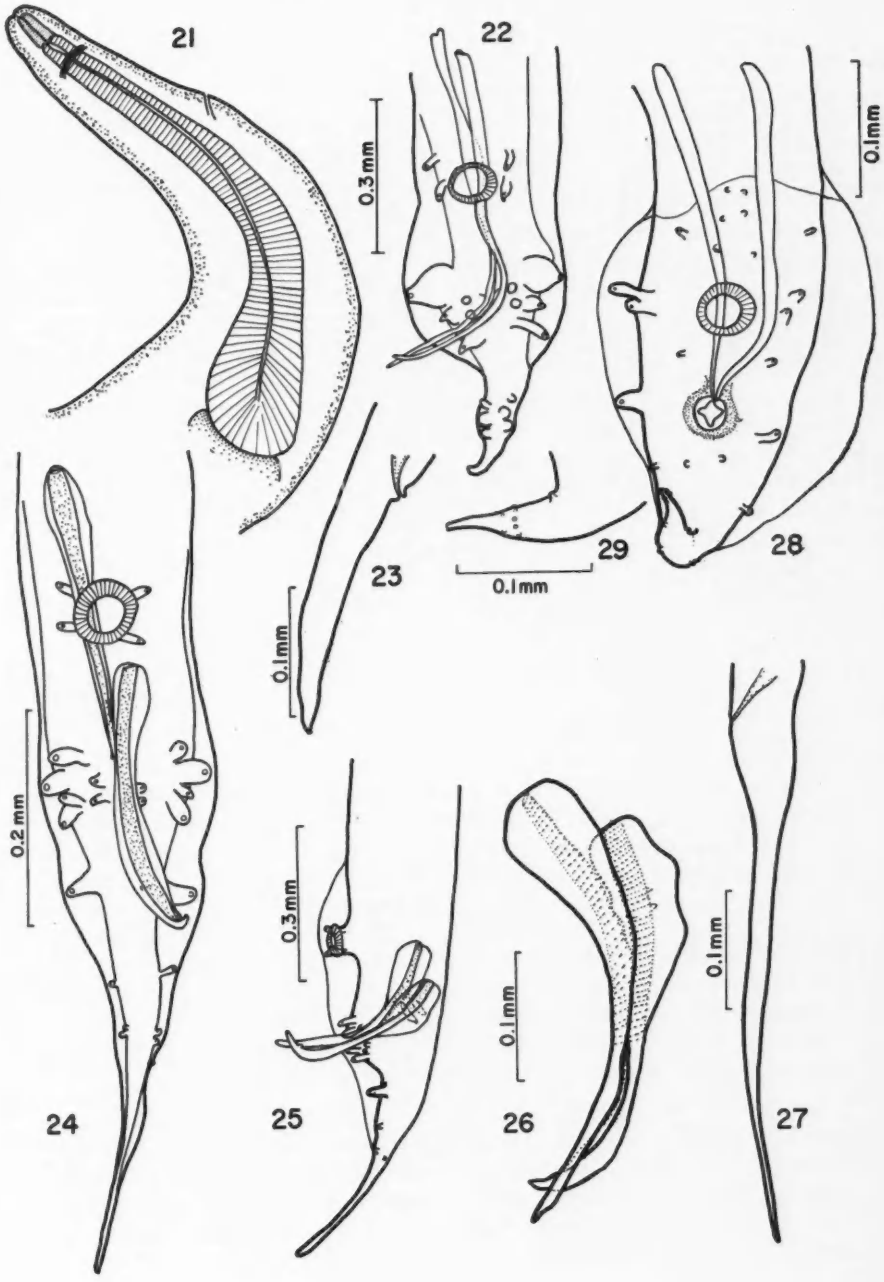
FIGS. 21–23. *Heterakis spumosa* (Schneider, 1866).
FIG. 21. Anterior region of male, lateral view. FIG. 22. Male tail, ventral view. FIG. 23. Female tail, lateral view.
FIGS. 24–27. *Heterakis beramporia* (Lane, 1914).
FIG. 24. Male tail, ventral view. FIG. 25. Male tail, lateral view. FIG. 26. Spicules. FIG. 27. Female tail, lateral view.
FIGS. 28–29. *Meteterakis govindi* (Karve, 1930) Inglis, 1957.
FIG. 28. Male tail, ventral view. FIG. 29. Male tail, lateral view.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ring encircles the narrow part of the oesophagus anterior to the excretory pore. Lateral alae are present about 0.15 to 0.2 mm in the male and 0.18 mm in the female from the anterior extremity and terminate at the level of the cloaca in the male and continue to the tip of the female.

Male.—6.8 to 9.85 mm long, 0.2 to 0.22 mm wide. Head 0.08 to 0.09 mm in diameter. Pharynx 0.06 to 0.08 mm long. Oesophagus including bulb 0.76 to 0.84 mm long; bulb 0.2 to 0.22 mm by 0.11 to 0.13 mm. Nerve ring 0.24 to 0.3 mm and excretory pore 0.35 to 0.41 mm from anterior end.

Tail 0.36 to 0.43 mm long. Caudal alae well developed. Preanal sucker 0.07 to 0.1 mm by 0.05 to 0.06 mm situated 0.55 to 0.6 mm from tip of tail and 0.18 to 0.21 mm from cloacal aperture. Ten pairs caudal papillae (Fig. 22) of which two pairs situated at sides of sucker, five pairs (three lateral and two subventral) from a paracloacal group, and three pairs on more slender portion of tail. The most anterior pair of the lateral papillae on each side is very large with a stout granular base, the middle pair is smaller but also has a thickened base while the most posterior pair is long but comparatively slender. Of the papillae in the tail region, the middle one is stoutest the other two pairs being slender. Spicules equal, tubular, and broader at anterior end gradually becoming narrower towards posterior end, longitudinally striated, and ending in simple points. They are 0.35 mm in length.

Female.—14.2 mm long, 0.42 mm wide. Head 0.09 mm in diameter. Pharynx 0.1 mm long. Oesophagus including bulb 1.62 mm long; bulb 0.18 to 0.17 mm. Nerve ring 0.21 mm and excretory pore 0.42 mm from anterior end.

Tail (Fig. 23) 1.02 mm long. Vulva a little anterior to mid-body, 6.4 mm from anterior end. Lips of vulva slightly prominent. Eggs, with mamillated shells, measure 0.06 to 0.08 mm by 0.04 to 0.05 mm.

Discussion

The present form belongs to *H. spumosa* (Schneider, 1866) (4) but differs from some previous descriptions of this species in having spicules of equal length, in the position of the vulva (which is a little anterior to mid-body), in having a larger tail, and in the absence of papillae on the female tail. These differences, however, are considered variations within the species.

Heterakis beramporia (Lane, 1914) (Figs. 24–27)

Host: Domestic fowl.

Location: Small intestine.

Material: One slightly damaged female and three males.

Discussion

The present form belongs to *H. beramporia* (Lane, 1914) (15) but differs from the original description in the relative size of the spicules and in having the left spicule (Figs. 24–26) nonalate at the tip, in the position of the vulva (which is slightly anterior to mid-body), and in having a larger tail (Fig. 27). These differences, however, are probably variations within the species (see Table I).

TABLE I

Measurements of *Heterakis beramporia* (Lane, 1914) (in mm)

	Author's specimens		Lane (15)	
	Male	Female	Male	Female
Length	3.55 to 6.39	5.55	5 to 6	6 to 8
Maximum width	0.21 to 0.25	0.44	0.26	0.3
Length of tail	0.36 to 0.45	0.94	0.37	0.66
Length of spicules				
Right	0.32 to 0.37	—	0.35	—
Left	0.27 to 0.32	—	0.3	—
Distance from head to vulva	—	2.12	—	About mid-body

Heterakis gallinae (Gmelin, 1790) Freeborn, 1923

Five male and eight female specimens were recovered from the small intestine of the domestic fowl from Dacca, East Pakistan.

Meteterakis govindi (Karve, 1930) Inglis, 1957 (Figs. 28–29)

Host: Bufo melanostictus.

Location: Small intestine.

Material: Seven females and five males.

Description

The worms are of medium size and taper towards each extremity. The vulva is covered by a prominent flap and lies from the anterior third to mid-body. The excretory pore opens into a large, lobulated excretory vesicle. The body bears numerous small sessile papillae on its surface. Narrow lateral alae terminate at the level of the sucker in the male but continue to the tip of the tail in the female.

Male.—5.02 to 5.4 mm long, 0.31 to 0.32 mm wide. Head 0.05 mm in diameter. Pharynx 0.06 to 0.07 mm long. Total length of oesophagus 0.85 to 0.92 mm, bulb 0.15 to 0.22 mm by 0.1 to 0.16 mm. Nerve ring situated 0.23 to 0.29 mm, excretory pore 0.42 to 0.44 mm from anterior end. Tail 0.15 to 0.17 mm long, curled ventrally (Figs. 28–29) ending in fine, sharply pointed spike. Sucker 0.06 to 0.07 mm from cloacal aperture, measuring 0.04 to 0.05 mm in diameter. Spicules 0.27 to 0.42 mm long.

Female.—5.78 to 7.77 mm long, 0.32 to 0.4 mm wide. Head 0.05 to 0.06 mm in diameter. Pharynx 0.06 mm long. Oesophagus 0.78 to 0.92 mm long; bulb 0.17 to 0.22 mm by 0.14 to 0.18 mm. Nerve ring 0.26 to 0.38 mm, excretory pore 0.38 to 0.56 mm from anterior end. Tail long, pointed, 0.25 to 0.36 mm long. Lips of anus raised prominently; vulva a little anterior to mid-body, 2.14 to 3.55 mm fron anterior end. Eggs 0.05 to 0.07 mm by 0.04 to 0.05 mm.

Discussion

The genus *Meteterakis* was established for *M. govindi* by Karve (13) but was subsequently considered invalid. As a result, *M. govindi* has since been described several times under different names; these are reviewed in detail by Inglis (10), who considers *Meteterakis* a valid genus and created a new subfamily, Meteterakinae, for its reception. The subfamily is characterized by

no interlabia, caudal alae fairly prominent and supported by three or four large fleshy papillae, a large number of small sessile papillae on the tail, and a vulva covered by a flap developed from the anterior lip of opening. Inglis (9, 10) is of the opinion that in the genus *Meteterakis* there is an indefinite gubernacular mass developed from the wall of the cloaca. He (10) regarded *M. govindi* Karve, 1930 (13), *Africana varani* Maplestone, 1931 (17), *Spinicauda bufonis* Yamaguti, 1935 (26), *Heterakis govindi* Baylis, 1936 (4), and *Ganguleterakis govindi* Skrjabin, 1949 (19) as synonyms. I agree with the reconstitution of the genus *Meteterakis* and with the synonymy of the above-mentioned forms, but am doubtful of the taxonomic value of the gubernacular mass.

The forms described in this paper resemble closely *Meteterakis govindi* as described by Karve (13), Koo (14), and Inglis (10) except in the number and arrangement of the caudal papillae, in the structure and size of the spicules, in the possession of a rather amorphous mass somewhat similar to the gubernacular mass of Inglis, and in the position of the vulva.

In the present form (Figs. 28–29) there are 15 pairs of cloacal papillae, 4 pairs small, subventral, situated in front of the sucker, and 3 pairs at the sides of the sucker. The anterior two pairs are fleshy and the posterior pair very small. Three pairs of cloacal papillae, one pair at the sides of the cloaca are large and lateral, two pairs in front of the cloaca are small and subventral. Five more pairs of small ventral papillae are mostly subventral, situated along the tip. In addition to the papillae mentioned, the area surrounding the cloacal aperture is covered with minute papillae. Anterior to the caudal papillae the cuticle is covered with numerous papillae throughout the body length. Karve (13) described 17 pairs of caudal papillae while Inglis (10) was of the opinion that there are 13 pairs on the tail and 7 pairs on the ventral surface of the body anterior to the caudal alae. The author is in agreement with Koo (14) and Inglis (10) that the number and arrangement of caudal papillae are variable except for the three large fleshy papillae, two suctorial, and one cloacal. Karve (13) appears to have overlooked seeing the papillae anterior to the caudal alae.

In the present form the spicules (Fig. 28) are equal, similar, tapering, and very delicate distally. The spicules differ from the forms described by others in being longer—0.27 to 0.42 mm long instead of 0.27 mm long in Karve's specimens, 0.24 to 0.27 mm long in Koo's specimens, and 0.18 to 0.27 mm long in Inglis' specimens, and being nonalate can be distinguished from the forms described by Inglis (10).

Inglis (10) is of the opinion that a gubernacular mass about 0.11 mm long is present. Furthermore, he stated that Karve appears to have over-cleared his specimens and failed to note the gubernacular mass. In the present form a rather amorphous mass (Fig. 28) somewhat similar to the gubernacular mass of Inglis was observed but was so ill-defined as to be questionably a distinct structural entity—at least in the author's mind.

In the present form the vulva lies about from the anterior third to mid-body while in the forms described by Karve, it is 2.2 to 2.8 mm, in Koo's specimens

2.12 to 3.2 mm, and in Inglis's 2.2 to 3.2 mm near mid-body or slightly anterior to it.

The position of the excretory pore, which opens into a large lobulated excretory vesicle, is similar to that described by Inglis but not mentioned by Karve.

These differences are considered variations within the species.

References

1. Ballesteros Marquez, A. Revisión de la familia Cosmocercidae Travassos, 1925. Rev. ibérica parasitol. 150–180 (1945).
2. Baylis, H. A. On two new species of *Oxysomatium* (Nematoda), with some remarks on the genus. Ann. Mag. Nat. Hist. 9 s. (110) **19**, 279–286 (1927).
3. Baylis, H. A. Some parasitic nematodes from the Uluguru and Usambara Mountains, Tanganyika Territory. Ann. Mag. Nat. Hist. 10 s. (22) **4**, 372–381 (1929).
4. Baylis, H. A. The fauna of British India including Ceylon and Burma. Nematoda, I. (Ascaroidea and Strongyloidea). Secretary of State for India in Council, London. 1936.
5. Baylis, H. A. and Daubney, R. Report on the parasitic nematodes in the collection of the zoological survey of India. Mem. Indian Museum, **7**, 263–347 (1922).
6. Baylis, H. A. and Daubney, R. A further report on parasitic nematodes in the collection of the zoological survey of India. Record Indian Museum, **25**, 551–578 (1923).
7. Baylis, H. A. and Daubney, R. A synopsis of the families and genera of Nematoda. British Museum, London. 1926.
8. Hsü, H. F. Some species of *Porrocaecum* (Nematoda) from birds in China. J. Parasitol. **19**, 280–286 (1933).
9. Inglis, W. G. A review of the nematode superfamily Heterakoidea. Ann. Mag. Nat. Hist. 12 s. **10**, 905–912 (1957).
10. Inglis, W. G. A revision of the nematode genus *Meteterakis* Karve, 1930. Parasitology, **48**, 9–31 (1958).
11. Jorge da Silva, A. A. Nova espécie do gênero *Aplectana* Railliet and Henry, 1916 (Nematoda, Cosmocercidae). Mem. inst. Oswaldo Cruz, **52**, 415–418 (1954).
12. Karve, J. N. A redescription of the species *Oxysomatium macintoshi* (Stewart, 1914) (Nematoda). Ann. Mag. Nat. Hist. 9 s. **20**, 620–628 (1927).
13. Karve, J. N. Some parasitic nematodes of frogs and toads. Ann. Trop. Med. Parasitol. **24**, 481–491 (1930).
14. Koo, S. Y. Nematodes parasites of *Bufo melanostictus*, the common toad, from Canton. Lingnan Sci. J. **18**, 143–154 (1939).
15. Lane, C. Suckered round-worms from India and Ceylon. Indian J. Med. Research, **2**, 655–669 (1914).
16. Lent, H. and Freitas, J. F. T. de, Uma coleção de Nematódeos, parasitos de vertebrados, do Museu de Historia Natural de Montevideo. Mem. inst. Oswaldo Cruz, **46**, 1–71 (1948).
17. Maplestone, P. A. Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens. Parts 4–8. Record Indian Museum, **33**, 71–171 (1931).
18. Railliet, A. and Henry, A. Nouvelles remarques sur les oxyuridés. Compt. Rend. **79**, 247–250 (1916).
19. Skrjabin, K. I. and Shikhobalova, N. P. Parasitic nematodes and diseases caused by them. Vol. 1. Oxyurata. (In Russian.) Moscow. 1949.
20. Skrjabin, K. I., Shikobalova, N. P., and Mozgovoi, A. A. Descriptive catalogue of parasitic nematodes. Vol. 2. Oxyurata and Ascaridata. (In Russian.) Izdatelstvo Akad. Nauk SSSR, Moscow. 1951.
21. Travassos, L. Pesquizas helminthologicas realizadas em Hamburgo. IX. Ensaio monographico da familia Cosmocercidae Trav., 1925. (Nematoda). Mem. inst. Oswaldo Cruz, **25**, 237–298 (1931).
22. Walton, A. C. Studies on some nematodes of North American frogs. I. J. Parasitol. **15**, 227–240 (1929).
23. Walton, A. C. The nematoda as parasites of amphibia. J. Parasitol. **20**, 1–32 (1933).
24. Walton, A. C. Notes on amphibian parasites. Proc. Helminthol. Soc. Washington, **7**, 87–91 (1940).
25. Walton, A. C. The finer structure of *Aplectana hamatospicula* (Nematoda). Proc. Helminthol. Soc. Washington, **8**, 18–21 (1941).
26. Yamaguti, S. Studies on the helminth fauna of Japan. Part 10. Amphibian nematodes. Japanese J. Zool. **6**, 387–392 (1935).
27. Yamaguti, S. Studies on the helminth fauna of Japan. Part 36. Avian nematodes. II. Japanese J. Zool. **9**, 441–448 (1941).

ON THE MORPHOLOGY AND LIFE HISTORY OF PHOCANEMA DECIPIENS (KRABBE, 1878) MYERS, 1959 (NEMATODA:ANISAKIDAE)[1]

B. J. MYERS

Abstract

Phocanema (synonyms: *Porrocaecum, Terranova*) *decipiens* is described in detail and its probable life cycle outlined. Eggs deposited in salt water develop and hatch in 7 to 14 days at between 10° C and 24° C, even after previous freezing. Temperatures over 24°C are lethal. Larvae fed to a large variety of invertebrates passed quickly through their intestines still alive; fed to fish, they disappeared within 24 hours but in one case a larva was found ensheathed in the intestine. No larvae were found in 'wild' invertebrates although many were infected with free-living nematodes. It is concluded that, while numerous invertebrates may act as 'transport' hosts for the larva to a fish, none acts as a true intermediate host. While larvae infective to seals occur commonly in the muscles of cod, a large variety of other fish are also infected and are a more probable source of infection. Development to maturity in the seal takes approximately three weeks, and it is probable that the main source of the infection in the Gulf of St. Lawrence is the harp seal, although harbor and grey seals also contribute to it.

Introduction

Phocanema decipiens (Krabbe, 1878) Myers, 1959,[2] a member of the family Anisakidae Skrjabin and Karokhin, 1946, subfamily Anisakinae Railliet and Henry, 1912, occurs as an adult in the stomach of numerous species of seals. Because it is a common parasite of grey, harp, and harbor seals in waters off the eastern Canadian coast a study has been made of its morphology and life history.

Adult Morphology

There are three lips with dentigerous ridges; interlabia are absent. Each lip is marked by a median indentation giving it a bilobed appearance. The dorsal lip is constricted at its base and bears two papillae just above the constriction, while the two subventral lips have a single medially placed papilla. The cuticle is conspicuously striated. Cervical alae are absent. The nerve ring and the rounded cervical papillae lie at the same level. The oesophagus is composed of an anterior preventricular region and a posterior ventricular region. An intestinal caecum arising from the intestine extends anteriorly along the oesophagus. A long ribbon-like gland is located on the left side of the anterior third of the body and opens to the exterior by a pore at the base of the subventral lips. Adult males measure from 25 to 70 mm in length and 1 to 1.5 mm in width. The tail is bent ventrally and terminates in a small spined process. Caudal alae are present. There are 50 to 100 pairs

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[2]Synonyms: *Porrocaecum, Terranova.*

of preanal and 6 pairs of postanal pedunculate papillae; the third pair of postanal papillae is the largest and has a double ending. A single sessile, medial, subterminal papilla is present and a pair of phasmids lie posterior to the last pair of postanal papillae. Three dentate ridges are seen posterior to the cloacal opening. The spicules are nearly equal, 1.5 to 2.5 mm in length, and terminate in a hook. The adult females measure from 35 to 80 mm in length and 1.5 to 2 mm in width. The vulva is situated in the middle third of the body. The eggs are spherical, 48 to 56 μ in diameter.

Cuticle and Hypodermis

The body is covered with a non-cellular cuticle beneath which lies the thin hypodermal layer, which is differentiated into four longitudinal chords—two lateral, a dorsal, and ventral—which lie between the muscle cells; posteriorly the dorsal and ventral chords are poorly developed and tend to disappear. When examined in cross section the cuticle is seen to be composed of an external, an internal cortical, and an oblique fiber layer, the internal matrix, and a basal lamella. These layers are the same as those described by von Linstow (5). The coelomocyte cells are found in the anterior region of the body and appear as a chain of independent cells. Branched cells, as described for *Contracaecum*, were not observed.

Musculature

The individual coelomyarian muscle cells are rhomboidal in shape and composed of a fibrillar zone extending up the sides of the cell, and a protoplasmic zone containing the nucleus. Somato-oesophageal muscle extends from the oesophagus to the body wall and somato-intestinal muscles from the intestine to the body wall. The depressor ani muscle is H-shaped and extends between the dorsal wall of the rectum and anus and the dorsal lateral side of the body. A rector spiculi muscle extends from the head of the spicule to the lateral chord and the protractor spiculi from the spicule to the ventral side of the body.

Excretory System

The asymmetrical system consists of a terminal duct and gland with a lateral canal in the left chord. Histological studies show the excretory pore opening between subventral lips as a slit, which connects to a duct the walls of which show cellular divisions. In cross section this wall appears as a layer of dark-staining material. Since the covering of the excretory gland is a syncytial cytoplasm devoid of nuclei, the cells may be epidermal cells which have become invaginated to form a lining for the duct. The ribbon-like excretory gland lies in the perienteric region of the left side of the anterior third of the body. The lumen widens to form an excretory sinus in which there is a single nucleus. Many fine branches connect with the lateral chord. Posteriorly, the gland attaches to the left lateral chord.

Nervous System

The nervous system consists of a circumoral fibrous ring and ganglion surrounding the oesophagus. Associated with the nerve ring are lateral and

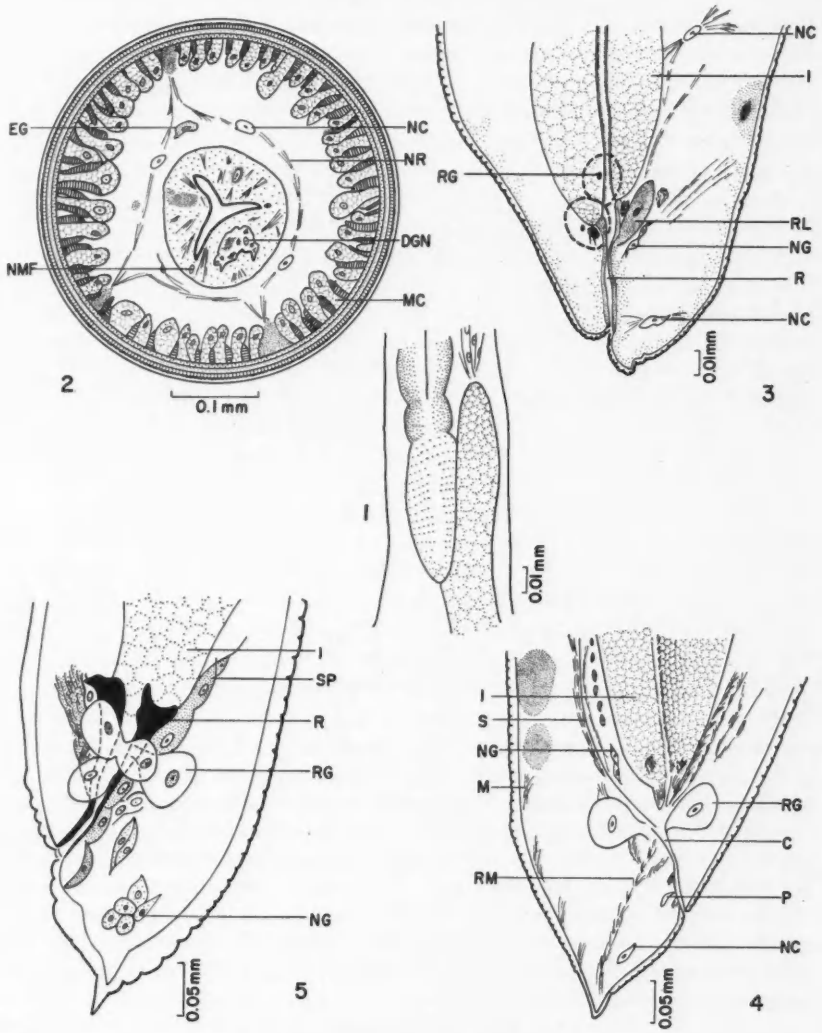

FIG. 1. Oesophageal region. FIG. 2. Cross section of oesophageal region. FIG. 3. Longitudinal section, female tail. FIG. 4. Longitudinal section, male tail (adult). FIG. 5. Longitudinal section, male tail (developing adult).

ABBREVIATIONS:

C, cloaca
DGN, dorsal gland nuclei
EG, excretory gland
I, intestine
M, muscle
NC, nerve cell
NG, nerve ganglion
NMF, nuclei marginal fiber
NR, nerve ring
P, papillae
R, rectum
RG, rectal gland
RL, rectal ligament
S, spicule
SP, spicular pouch

ventral ganglia and the papillary nerves which extend forward to the papillae. Posteriorly, there is a middorsal nerve which arises from the lateral ganglion. A branch from this nerve extends forward to supply the cervical papillae. A detailed study of the posterior and anterior nervous system was not attempted.

Digestive System

Oesophagus

The oesophagus, which appears to be divided into two portions, has a triradiate lumen, one ray of which is directed ventrally, the other two dorsally. The anterior preventricular region extends posteriorly from the lips to the ventil muscles and the ventriculus extends from the ventil muscles to the intestine. Anteriorly the ventriculus becomes dilated to form a bulbular portion. Although Cobb (1) considered this bulbular portion to be a separate region, it is not histologically different from the ventriculus proper. There are radial and marginal oesophageal muscle fibers: the former are numerous, running from the lumen to the sides, and the latter originate at the arms of the triradiate lumen. A ramified dorsal gland arises in the ventriculus, narrows at the level of the preventriculus, and extends forward to approximately the level of the nerve ring, where it opens as a narrow slit into the lumen of the oesophagus. The nucleus of the dorsal gland is curved and lies in the posterior portion of the gland. A subventral gland and two nuclei are observed in the ventriculus. The presence of the two nuclei supports Hsü's (2) observation although Jägerskiold (4) thought the nuclei were degenerate. The oesophagus communicates with the intestine by an oesophageal–intestinal valve.

Intestine

The intestine is an epithelial-lined tube with an outer tunica propria covering. In the ventricular region (Fig. 1) the intestine gives rise to an anteriorly projecting intestinal caecum. The caecum in cross section appears to be composed of "palmate" structures without definite cellular boundaries, arranged radially to form an irregular lumen. The caecum is supported by fibers which terminate in or near the lateral chord. The function of the caecum is unknown. In the female the intestine opens ventrally to the anus. In the male the reproductive system joins the rectum to form a cloaca. The cuticularly lined rectum has three large rectal glands in the female and six to eight in the male. An anal sphincter is present.

Reproductive System

The reproductive system is of the telogonic type, that is, the germ cells proliferate in the proximal portion of the gonad—the germinal zone—which is succeeded by a growth zone where the germinal cells enlarge and become differentiated into gametes.

Male

There is a single testis, the proximal portion of which is covered by a cap cell of epithelial tissue. The spermatogonia form a solid column and as they increase in size they become arranged radially around a rachis. The seminal

vesicle is a thin-walled sac in which the epithelial cells are squamous in the anterior portion and columnar in the posterior portion. Posteriorly the seminal vesicle narrows to form a canal, opening into a dilated glandular vas deferens which terminally becomes a chitinous walled rectum opening ventrally into the cloaca. The posterior part of the anal sphincter muscle surrounds the ejaculatory duct. Three large ovoid rectal glands are present connected with a broad band-like rectal ligament.

Two subequal spicules each terminate in two phalanges. The proximal end of each spicule is semilunar in shape. Each spicule contains a protoplasmic core and is surrounded by a cuticle which is continuous with the cuticular lining of the spicular canal. The outer layer appears colorless and is loose-fitting, whereas the inner layer is darker and well defined. No nuclei were observed within the protoplasmic core. Two sets of muscles control the spicules: the retractor muscles, which attach to the proximal end of the spicules and the protractor muscles, which insert posteriorly to the body wall. The spicules lie in a spicular pouch. Posteriorly, at the level of the rectal ligament, the spicules enter a chitinized spicular canal; the two canals at first are separate but unite to form a common canal which opens into the cloaca as a dorsal slit. A gubernaculum is absent.

Female

The ovaries are tubular sacs covered by a simple multinucleated epithelium, which is very thin in the terminal region. The blind end of the sac is covered by an apical cell. The germinal zone is represented by many small cells crowded so closely together as to make their boundaries difficult to distinguish. These cells divide mitotically to produce oögonia, and a rachis is formed. The rachis appears as a central strand of non-nucleated tissue extending to the beginning of the oviducts. The oögonia become "tear-shaped" and are arranged radially around the rachis. The ovaries open into narrow, non-muscular tubular oviducts lined with columnar epithelium. Near the terminal portion of the ovaries the oögonia drop from the rachis and become tightly packed in the oviducts where they assume the typical round shape. There is no definite seminal receptacle, the region where the uteri and the oviducts unite serves this purpose. In this region oblique and circular muscles are observed. The seminal receptacle may be occluded in older females. Epithelial cells of this region have become long and form a plug almost closing the uterus proper. Sperm cells can only be observed when ova are absent.

The uteri are lined with squamous epithelial cells. The size and shape of the uteri is dependent upon their condition; when they are filled with eggs they become distended and when empty, completely compressed. Distally the uteri enter a common muscularized tubular vagina. The columnar epithelial cells of the uteri are continuous with those of the vagina but sometimes, due to contraction, appear to be cuboidal. These cells form an irregular lumen which is usually narrow, allowing the passage of only a single egg. An anastomosing circular and oblique muscle layer surrounds the vagina, and a connective tissue membrane serves as an external covering. The vagina is divided into

two portions: a vagina uteri which is the portion preceding the uterus, and a vagina vera which has a cuticular-lined lumen formed by the cuticle of the vulva. The cells of the vagina vera are larger and more numerous than those of the vagina uteri.

The Egg

The egg is oval in shape and varies in size from 48 to 54μ in diameter. The external covering is smooth and possesses adhesive properties. A chitinous membrane (the shell) and a vitelline membrane comprise the egg coverings.

The Life Cycle

The egg is in the morula stage when passed in the faeces of the seal and certain conditions are necessary for its development to a larva. Although it is impossible to duplicate natural conditions in the laboratory, attempts were made to determine the effects of temperature on the development of the egg.

Eggs were obtained by dissecting the vagina and uteri of adult worms collected from seal stomachs and their stage of development varied from the uncleaved to the morula.

The seal spends most of its life in sea water the temperature of which varies both seasonally and geographically. Eggs which are passed in its faeces must, therefore, be able to withstand these variations. In the laboratory it was found that for development and hatching of the egg, the optimum temperature varied between 10° and 24° C. Even when subjected to previous freezing the egg developed and hatched in from 7 to 10 days. These findings confirmed those of Scott (11). Below the minimum level, development was retarded. At 4° C there was no development beyond the morula stage. Temperatures higher than 24° C were lethal.

Short periods of desiccation are lethal to the eggs and moisture is essential for hatching. The moisture content of the faeces is sufficient to maintain water constancy but prolonged drying on seal "hauling out" sites would kill the eggs.

Development and hatching will proceed in closed petri dishes. Hatching will occur in solutions of seal faeces. When treated with dilute formalin, potassium bichromate, or artificial digestive juices eggs will neither develop nor hatch.

The Larva

Within 7 to 14 days a fully formed, ensheathed larva is observed moving spasmodically within its flexible, transparent, very thin-walled shell. By repeated pushing—produced by coiling and uncoiling itself—the larva succeeds in breaking the egg shell medially and emerges head first. Still adhering by its tail to its castoff shell it thrashes about in the water. If freed from the shell, it will move independently but the thin, flexible tail will adhere to particles of debris and the movement in the water is limited to a thrashing one radiating from the attachment point of the tail. At 4° C the living larvae are seen in a coiled position; raising the temperature produces activation and the movement becomes typical.

Newly hatched larvae, 200 μ long, having molted once within the egg are in the second stage but still enclosed within the cast cuticle. The cuticle is very loose-fitting and at times—especially upon the death of the larva—appears to be lacking. A boring tooth is present. Cephalic structures are not fully formed and a straight, undifferentiated digestive tract is seen. Larvae sink to the bottom of the culture dishes where they remain. Although they have lived for 14 days in no case had the cuticle been shed or further development taken place.

Larvae will live for 11 days (pH 7.2 to 7.5) in a dish in which cod muscle has been placed, for 5 to 7 days in stagnant cultures (that is, with the water unchanged), for 10 to 14 days in sea water, and for 2 days in seal faeces. Placement in artificial digestive juices, freezing, and drying killed the larvae. At 4° C larvae will remain alive although inactive for 30 days.

In order to become sexually mature and complete their life cycle, larvae must return to the seal. Schiffman (personal communication) and Scott (10) have shown that a stage infective to seals occurs in the musculature of a variety of marine fishes.

The remaining gap in the life cycle was the method whereby the second-stage larva from the egg reached the fish. It appeared that either a specific intermediate host—in which some essential development took place—or a merely passive carrier host would be required.

Eggs deposited in seal faeces in the sea are, as has already been mentioned, subjected to various environmental conditions and may pass through pelagic and benthonic zones. Accordingly, all invertebrates living in these zones could be considered as potential hosts—either intermediate or carrier.

Once in the sea, depending upon the degree of action of waves, currents, and density, the faeces will disintegrate and the eggs be deposited at various depths and, unless consumed on the way, will eventually reach the bottom.

If the eggs are consumed by invertebrates in the pelagic zone, their rapid metabolism could allow the eggs to pass through with little or no change. The body temperature of the invertebrate has the same range as its environment. An egg in the morula stage requires 7 days to embryonate at 24° C, 14 days at 14° C. Accordingly, if a pelagic invertebrate is the intermediate or carrier host it is probable that the larva rather than the egg is the infective stage.

If, on the other hand, the eggs reach the bottom and if the temperature of the environment is suitable for development, the eggs will hatch and the larvae may:

(1) be consumed by a filter feed (invertebrate),

(2) be consumed by a scavenger (invertebrate or vertebrate),

(3) be consumed by an invertebrate or vertebrate which is itself consumed by a predator, or

(4) be consumed by a fish.

If the temperature is unfavorable for development the egg may remain in the morula stage until the temperature is favorable.

Field Studies

During the summers of 1953 to 1956, inclusive, investigations were conducted at the Fisheries Research Board of Canada Biological Station, St. Andrews, N.B.; the Marine Biological Station, Grande Rivière; and the Marine Biological Laboratory, Grindstone, Magdalen Islands, of the Quebec Department of Fisheries.

At sea, small beam trawls, small bottom dredges, and other modified gear were used to collect material. In the intertidal, tidal, and littoral zones material was collected by hand, with nets, and with seines. Surface and bottom temperatures were recorded.

Specimens to be used for experimental infections were transferred to aquaria equipped with a continuous flow of salt water, and aerated. With experience, it was possible to learn the methods necessary to keep the various invertebrates alive under laboratory conditions. Their feeding habits, where possible, were studied and experimental infections adjusted to these. Following infection with *Phocanema decipiens* larvae the invertebrates were held for 3 weeks, then dissected under a dissecting microscope and the various parts of the body studied under a compound microscope. The material was then washed from the slides into artificial digestive juices and the residue examined for the presence of larvae.

As many free-living nematodes resemble the hatched larvae of *P. decipiens* contamination with these was avoided by washing each invertebrate in distilled water before examination.

Table I records the invertebrates used for experimental infections. Controls were maintained in each experiment. Larvae survived in these invertebrates (except *Artemia salina*) and in amphipods, mysids, isopods, and fish as second-stage larvae for not more than 24 hours. Frequently, the larvae were passed alive in the faeces within 30 minutes of infection. The infection in amphipods and mysids has been transferred to fish but the larvae disappeared from the intestine of the fish within 24 hours. In a sculpin, however, still ensheathed living larvae were seen although there was no evidence of development.

Many small free-living nematodes living commensally in *Mysis mixta*, *M. stenolepsis*, *Crago septemospinosus*, *Pandulus borealis*, and *P. montagui* were collected; when first observed under the microscope these resembled the hatched larvae of *P. decipiens*. Closer examination showed them to be free-living nematodes which Steiner (12) described as a new species, *Monhystera cameroni*, a commensal of the above-mentioned hosts.

The Infective Larval Stage in Fish

Phocanema decipiens larvae were collected from witch (*Glyptocephalus cynoglossus* (Linnaeus, 1758)); from plaice (*Hippoglossoides platessoides* (Fabricius, 1780)) from North Bay, N.S.; and from cod (*Gadus callarias* Linnaeus, 1758) from Louisbourg, N.S., and Grande Rivière, Que. These larvae are relatively large and are in the stage infective to seals (10).

TABLE I

Phocanema decipiens infections

	Infections	
	Natural	Experimental
	No. specimens examined	
Annelids		
Nereis sp. (sandworms)	75	25
Sea mouse	20	3
Arthropoda		
Amphipods		
Anonyx nugax	10	—
Ampelisca sp.	80	50
Gammarus sp.	250	275
Onosimus plautus	6	—
Rachotropis aculeata	15	5
Syrrhoe crenulata	7	—
Cumacea sp.	20	10
Isopods		
Iodothea sp.	150	65
Mysids		
Mysis mixta Lilljebord	100	100
M. reticula Loven	50	25
M. stenolepsis Smith	50	50
Mysis sp.	250	35
Shrimps		
Argis sp.	25	25
Crago septemspinosus Say	150	300
Pandulus borealis Kröyer	25	10
Pandulus sp.	75	85
Spirontocaris groenlandica (Fabricius)	150	300
Crabs		
Cancer irroratus Say	30	40
Pagurus kroyeri Stimpson	25	26
Lobsters		
Homarus americanus	5	10
Mollusca		
Buccinum sp. (snail)	20	20
Littorina sp. (snail)	20	30
Mya areinaria L. (clam)	15	14
Mytilus edulis (blue mussel)	25	40
Echinodermata		
Starfish	25	30
Sea cucumber	20	20
Sea urchin	30	30
Teleosteii		
Pseudopleuronectes americanus (Walbaum) (flounder)	10	10
Gasterosteus aculeatus Linnaeus, 1758 (stickleback)	7	7
Osmerus mordax (Mitchell, 1815) (smelt)	2	2
Lebastis reticulatus (Peter) (guppy)	2	10
Myoxocephalus sp. (sculpin)	5	5

The larvae, which are yellow in contrast to the white of the fish fillet, are to be seen coiled or uncoiled in the fish muscle. Definite encystment of the larvae has not been seen and they appear to move freely within the muscle, particularly if heat is applied. Hard brown cysts containing unidentifiable caseous material are often found within the fish muscle; these may be due to a

host reaction on the death of *P. decipiens* larvae or to the larval stage of the cestode *Grillotia erinaceus* (van Beneden) Dollfus, 1942, which has been described as causing such cysts.

The larva has three bilobed lips, a dorsal one with two papillae and two subventral, each with a single median papilla. Dentigerous ridges are not developed. A characteristic boring tooth is situated near the ventral margin of the dorsal lip but does not appear to be derived from the lip pulp. The cuticle is transversely striated. Four longitudinal chords are present. The nervous system is poorly developed and appears to be confined to a fibrous ring surrounding the oesophagus. The body is composed of a fenestrated membrane of nucleated cells. A clear orange to red-colored fluid fills the perienteric region of the living larvae. The oesophagus is divided into an anterior or proventricular region and a posterior or ventricular one. The ventricular portion opens directly into the intestine. A caecum arises from the intestine and extends anteriorly along the lateral margins of the oesophagus and is attached to the body wall by a ligament. Posteriorly, the intestine ends in a cuticularly lined rectum to which are attached rectal cells. The excretory or ventral gland consists of a single ribbon-like gland lying in the perienteric region ventral to the intestine. This gland is composed of spongy cytoplasm and two canals, one anterior and the other attached to the left lateral chord posteriorly. The anterior canal extends forward and opens as a slit between the two subventral lips. The opening appears to be guarded by a small finger-like projection. The genital primordial is visible as a group of small rudimentary cells. The sexes cannot be distinguished.

The presence of larvae in the muscles of fish is governed by the distribution of the fish and by its diet. The cod, which is commonly infected, is non-selective in its feeding habits. When able it will feed exclusively upon schools of smaller fish but if these are not available it will feed on invertebrates that may be present. Inshore cod tend to be more heavily infected than offshore cod and this fact is correlated with the distribution of seals and to the higher water temperatures which favor development and hatching of the nematode eggs. In summarizing the movement of cod in eastern Canadian waters Mackenzie (6) stated:

> "Movement of the cod was related to submarine physiography, deep channels acting as barriers. Within the area as a whole, movement increased from west to east as does the seasonal range of water temperature. Larger cod moved on an average more than the smaller. Average size of cod increased with depth. Cod of the same size from deeper water moved more than those from shallower water. Movements have been related to spawning in some instances. No large scale well defined movement related to feeding was shown, although concentrations of food are believed to influence movement. Seasonal changes in local fisheries for cod were related to movement indicated by tagging."

The movement of cod from the Nova Scotia region into the Gulf of St. Lawrence and the consequent mixing of cod populations each with a certain

index of infection make it difficult to establish all-over incidence figures for the Gulf. Because seals are not selective in their food habits and cod is not a major item in their diet, it follows that other species of fish must frequently carry the infection to them.

Templeman, Squires, and Flemming (13) have studied the infection in other fish in Newfoundland. They found that on a weight basis smelt are heavily infected although on an individual basis, the infection seems light. Smelt are inshore fish, prefer a high surface temperature, show little movement except that needed for spawning, and feed upon crustacea, small fish, and polychaete worms. Tomcod which share similar habits with the smelt are very lightly infected. Haddock are lightly infected but as they live offshore in deeper water perhaps have less opportunity of becoming infected. Pollock also are lightly infected and feed mainly on pelagic fauna. These authors found witch, yellowtail, and winter flounder—which consume very few fish—to be lightly infected whereas the American plaice—a fish feeder—is heavily infected, even although the latter occur in shallow water. Herring, capelin, and other pelagic fish were found to be free of infection; longhorned sculpin, angler, Greenland halibut, Greenland cod, pollock, yellowtail, sea raven, and red fish were infected.

Small encysted *Phocanema*-type larvae were collected by the author from the stomach wall of skates (*Raja* sp.) from the Magdalen Islands, Que. These larvae had small, developing intestinal caeca and differed from the larvae collected from fish musculature mainly in size.

There are various *Ascaris*-type nematodes in marine animals in the Gulf of St. Lawrence, and *P. decipiens* can be distinguished from others mainly by the presence of an intestinal caecum. However, there are others, particularly *Anisakis*, in which there is no caecum. In all genera, when the young larvae hatch from the egg there is no visible intestinal caecum and as a consequence, except in controlled experiments, Phocanema-type larvae can be recognized with certainty only after development of the caecum and it is unknown when this development takes place. Very small forms without a caecum may belong to any genus and are referred to as *Anisakis*-type larvae. The reproductive and nervous systems are the last to develop whereas increase in size and growth of the digestive tract and excretory systems occur earlier. The distinctive cephalic structures are formed only after the final molt in the seal stomach and consequently recognition of the early larval stages must be based on size and the morphology of the digestive tract.

The larval stages of the Anisakinae have been reported from cod and various ground fish in North American and other regions. These larvae were recognized as early as 1767 and approximately 85 larval forms occurring in fish have been reported, described, and assigned scientific names. It is impossible to attempt to recognize these larval stages on the basis of early descriptions and they must be regarded as *nomina dubia*.

In the structure of the digestive tract larval stages of Anisakinae found in fish and invertebrates resemble the adult stages but the cephalic structures

are only evidenced by the presence of three lips. As many genera possess the same type of digestive tract it is difficult if not impossible to assign the larval stages to genera. Accordingly, I propose the following names, based on digestive tract formation, for the larval stages of the Anisakinae:

(1) Phocanema type: ventriculus present. Anteriorly projecting intestinal caeca present.
(2) Anisakis type: ventriculus present. Anteriorly projecting intestinal caeca absent.
(3) Contracaecum type: ventriculus and ventricular appendix present. Anteriorly projecting intestinal caeca present.
(4) Raphidascaris type: ventriculus and ventricular appendix present. Anteriorly projecting caeca absent.

Both Phocanema-type and Anisakis-type larvae were found in the length class of 17 to 34 mm. Templeman, Squires, and Flemming (13) reported no Anisakis-type larvae longer than 34 mm. It is possible that the young forms lacking a caecum were Phocanema-type larvae in which the intestinal caecum had not yet developed.

The Adult Stage in Seals

The final larval stage, after an unknown number of molts in the stomach of the seal, emerges as a sexually mature adult in 21 days. The general health of a seal does not seem to be affected by massive infections.

As it is impossible to examine seals immediately after death one can only speculate as to the worm's typical method of attachment or its exact position in the body. Adults appear to be free in the lumen of the stomach and intestine although some have been observed attached to the stomach mucosa and to the intestinal wall.

After the death of the seal, worms have been seen escaping through the nostrils, mouth, and rectum. Due to the methods of capturing seals, it is usually 24 hours or longer before examination is possible so that it is impossible to determine accurately what worm population a seal harbored prior to its death. Moreover, the larvae and developing stages are identified with great difficulty since the cephalic structures are not formed at this time.

Anisakid nematodes in seals cannot be identified with accuracy superficially (that is, by gross morphology), nor, in the case of mixed infections, can the percentage of the various genera be estimated. It is necessary to expose the digestive tract of adult worms and to prepare *en face* views of heads of specimens possessing a Contracaecum-type digestive tract. As *Contracaecum* and *Phocascaris* possess the same type of digestive tract they can be separated only on the basis of cephalic structures. Furthermore, many fish upon which seals feed are infected with species of anisakid nematodes which may be transitory parasites in the seal. Identification of adults must be based on specific measurements and morphology.

All seals of the north Atlantic coast of Canada are potential hosts. The harbor seal—the numbers of which are estimated to be 15,000 (3)—is the

most widely distributed resident seal, being found throughout the year from the Bay of Fundy to the north shore of the Gulf of St. Lawrence. These seals are generally considered to be non-migratory although Fisher and Scott (2) observed local migrations in the Bras d'Or Lakes.

The grey seal is more locally distributed. It is estimated (3) that about 7000 occur in Canadian waters. Their principal area of concentration is in the southwestern area of the gulf, with a smaller herd of about 2000 at Amet Island in the Northumberland Straits, and another, of about 400, at Deadman Island, near the Magdalen Islands. Smaller concentrations also occur around Anticosti Island; at Grand Manan, Bay of Fundy; on the northeastern coast of Nova Scotia; in the Bras d'Or Lakes; and at Sable Island.

A population of harp seals (3), variously estimated to be between one and two million, enters the Gulf of St. Lawrence by the Strait of Belle Isle in January and, after whelping and mating, leaves again by the same Strait in May or June. From the limited data available (3) it is considered that they enter the Gulf apparently uninfected with *P. decipiens* but are infected when they leave.

Even although lightly infected the enormous number of harp seals concentrated in the Gulf must be considered a major factor in the dispersal of the eggs of *Phocanema*.

Conclusions

Larval stages of the various genera of the Anisakinae have been observed in the body cavity, mesenteries, and other organs of vertebrates and invertebrates, and there is a considerable variety of developmental patterns.

Thomas (14) found that the dragonfly nymph and guppy were utilized as intermediate hosts for *Raphidascaris canadensis* of the muskelunge. He also observed that the guppy could be infected directly with the embryonated eggs, the intervention of the nymph being unnecessary.

Thomas (15) experimentally completed the life cycle of *Contracaecum spiculigerum* of cormorants. The eggs hatched into larvae and were infective to guppies; after 3 months the larvae were observed in the mesenteries of the guppies. These larvae were infective to cormorants. However, if the infected fish was consumed by another fish, the larvae would re-encyst in the mesenteries.

Mozgovoi (8) found the earthworm to be the intermediate host for *Porrocaecum crassum* of ducks.

Walton (16) found *Rana* and *Siren* sp. to be the intermediate hosts for *Multicaecum tenicollis*.

Experimental evidence of all stages of the life cycle of *Phocanema* is not yet available but from evidence that is available it would seem that:

1. Eggs passed in seal faeces embryonate and after 10 to 14 days develop into second-stage larvae.
2. The hatched, ensheathed larvae are eaten by an invertebrate in which no development takes place (that is, the invertebrate is a carrier or transport host only).

3. The invertebrate is eaten by a fish in the stomach of which the larvae bore into the stomach wall, encyst, and develop (after ensheathment in the muscles) to the stage infective to seals. (This stage can probably occur in any species of ground fish feeding on the proper invertebrates and in an area in which seals have dispersed the eggs.)
4. The seal is infected by eating the fish infected with the larvae, which reach sexual maturity in the digestive tract of the seal.

References

1. COBB, N. A. Neueparasitische Nematoden (*In* Kükenthal Beiträge zur Fauna Spitsbergens). Arch. Naturgeschichte Berlin, 55, J, **1**, 149–159 (1889).
2. FISHER, H. D. and SCOTT, D. M. Incidence of the ascarid *Porrocaecum decipiens* in the stomach of three species of seals along the southern Canadian Atlantic mainland. J. Fisheries Research Board Canada, **15**, 495–516 (1958).
3. HSÜ, H. F. The oesophageal glands of nematodes. Lingnan Sci. J. **12**, suppl., 13–21 (1933).
4. JÄGERSKIÖLD, L. K. E. Beiträge zur Kenntnis der Nematoden. Zool. Jahrb. Jena, Abt. Anat. **7**, 449–532 (1894).
5. VON LINSTOW, O. F. B. Untersuchungen an Nematoden. Arch. mikroskop. Anat. **44**, 509–533 (1895).
6. MACKENZIE, R. A. Atlantic cod tagging off the southern Canadian mainland. Bull. Fisheries Research Board Canada, **105** (1956).
7. MONTREUIL, P. L. J. and RONALD, K. A preliminary note on the nematode parasites of seals in the Gulf of St. Lawrence. Can. J. Zool. **35**, 495 (1957).
8. MOZGOVOI, A. A. The biology of *Porrocaecum crassum*, a nematode of aquatic birds. (Russian text.) Trudy Lab. Gel'mint. Akad. Nauk, S.S.S.R. **6**, 114–125 (1952).
9. MYERS, B. J. *Phocanema*, a new genus for the anisakid nematodes of seals. Can. J. Zool. **37**, 459–464 (1959).
10. SCOTT, D. M. Experiments with the harbour seal *Phoca vitulina*. A definitive host of a marine nematode *Porrocaecum decipiens*. J. Fisheries Research Board Canada, **10**, 539–547 (1953).
11. SCOTT, D. M. On the early development of *Porrocaecum decipiens*. J. Parasitol. **43**, 321–322 (1955).
12. STEINER, G. *Monhystera cameroni* n. sp.—a nematode commensal of various crustaceans of the Magdalen Islands and the Bay of Chaleur (Gulf of St. Lawrence). Can. J. Zool. **36**, 269–278 (1958).
13. TEMPLEMAN, W., SQUIRES, H. J., and FLEMMING, A. M. Nematodes in the fillets of cod and other fishes in Newfoundland and neighbouring areas. J. Fisheries Research Board Canada, **14**, 831–897 (1957).
14. THOMAS, L. J. Life cycle of *Raphidascaris canadensis* Smedley, 1933, a nematode from the pike, *Esox lucius*. J. Parasitol. **23**, 572 (1937).
15. THOMAS, L. J. On the life cycle of *Contracaecum spiculigerum* (Rud.). J. Parasitol. **23**, 429–431 (1937).
16. WALTON, A. C. A suggested life cycle for *Multicaecum tenuicolle* (Rud., 1819) Walton, 1927. J. Parasitol. **23**, 537 (1936).

EXPERIMENTAL INFECTIONS OF CYCLOPID COPEPODS WITH TRIAENOPHORUS CRASSUS FOREL AND T. NODULOSUS (PALLAS)[1]

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Abstract

Coracidia of both *Triaenophorus crassus* and *T. nodulosus* were fed to 19 species of cyclopid copepods, and coracidia of one or the other were fed to 4 other species. Procercoids of both species of parasite developed in nine species of cyclopids. Three species were infected with *T. crassus* only and four with *T. nodulosus* only. Size or feeding habits of the specimens did not influence the ingestion of coracidia. The presence of non-feeding individuals may have influenced the infection rate of some species. Herbivorous species, carnivorous species, and species which did not become infected ate large quantities of coracidia.

Microcyclops varicans rubellus was the most easily infected cyclopid species in the experiments. It is probably an important host of *Triaenophorus* in North American lakes along with *Cyclops bicuspidatus thomasi*, *Cyclops brevispinosus*, and, in certain areas, *Cyclops scutifer*.

Introduction

The first parasitic stage in the postembryonic development of pseudophyllidean tapeworms occurs when the free-swimming larva or coracidium is eaten by a copepod. The embryonic larva or onchosphere loses its ciliated coracidial covering, burrows through the gut wall of its host, and develops into a solid procercoid larva. This portion of the life cycle was worked out for *Diphyllobothrium latum* by Janicki and Rosen in 1918 (Rosen (10)), and the similar life history of *Triaenophorus nodulosus* (Pallas) was described by Rosen in 1919 (10). Michajlow (4) described the development of the eggs, coracidia, and procercoids of *T. crassus* Forel. According to Miller (6, 7), *Cyclops bicuspidatus thomasi* Forbes is the principal first intermediate host of *Triaenophorus* in North America. *C. strenuus* is considered the most effective natural host by European workers.

The present study was undertaken as part of the research into the ecology of *T. crassus* currently being carried out by the Fisheries Research Board of Canada. Nearly 30 years ago, Michajlow (5) showed that several European species of the family Cyclopidae could act as first intermediate hosts of *T. nodulosus*. He felt that the successful development of the procercoid in the body cavity of a given species of cyclopid depended largely upon two factors: whether the digestive action of the copepod killed some or all of the coracidia and whether the procercoid could develop normally in the milieu provided by the haemocoele of the cyclopid. On this basis he considered

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C. bicuspidatus Claus, which is closely related to our North American subspecies, of secondary importance as a host, as many of the coracidia eaten were digested.

Since several European species of cyclopids were potential first intermediate hosts of *Triaenophorus*, the investigation of several North American species of cyclopids was expected to prove that more than one species would be infected. This study attempts to evaluate the importance of each of these species as natural hosts by considering its degree of infection and ecological relationships within the parasite's life cycle.

Materials and Methods

The basic procedure undertaken in this study consisted of placing uninfected cyclopids in contact with viable coracidia and examining them at some later date for the presence of normal procercoids.

Triaenophorus eggs were obtained from adult worms found in the intestines of pike during May and early June. The eggs hatch into free-swimming coracidia a few days after they are removed from the worms if they are cultured at room temperature. The coracidia are collected by pipette from the surface of jars containing hatching eggs. Since they live only about 48 hours, the supply of fresh coracidia limited the number of infections which could be carried out. In order to obtain coracidia over a longer period of time, jars of eggs were refrigerated. When the water covering the eggs was changed regularly to provide aeration and remove excessive growths of protozoans, sufficient quantities of coracidia could be obtained for periods up to 3 weeks or a month. Subsamples of the cultures were removed from the refrigerator and held at room temperature overnight to induce hatching.

The cyclopids to be infected were collected from various localities, chiefly from the vicinity of Toronto, Ontario, and Heming Lake, Manitoba. Most of them were isolated, identified to species, classified by stage of development and sex, and maintained individually in 1-in.-sq. staining dishes. Coracidia were then added to the dishes containing the individual cyclopids. Each animal was examined for the presence of procercoids a week after introduction of coracidia. This time lapse allowed the procercoids to develop. Before examination the cyclopids were anaesthetized in a 0.1% solution of MS-222, an anaesthetic widely used in fishery biology. The procercoids continued to pulsate in the cyclopid's body after the host was rendered inert, and thus were detected more easily. The number of infected cyclopids and the number of procercoids per cyclopid was noted. These individually reared cyclopids were kept and re-examined from time to time until their death.

A slightly different method was used to obtain larger numbers of infected animals. This consisted of adding relatively large numbers of copepods to a jar or dish and exposing them to quantities of eggs and coracidia. The results obtained from these mass cultures were not as exact as those from the infection of individual specimens, especially as we were not able to estimate the number of copepods that had died before examination.

Although a few experiments of this type were carried out at Heming Lake in 1953 and subsequent years, their results were inconclusive. The major part of the work was undertaken by the junior author at the University of Toronto in 1957 and by the senior author at Heming Lake in 1958 and 1959.

Several experiments were carried out at Heming Lake in 1959 to determine the number of coracidia eaten by individuals of several species of cyclopids. In these experiments several cyclopids were introduced into a counting cell containing a known number of coracidia. After a given interval, usually 8 hours, but upon one occasion 2 days, the culture was anaesthetized, the cyclopids removed to separate dishes for future examination, and the number of uneaten coracidia recorded.

Nomenclature

The nomenclature used in describing the cyclopid copepods in this paper follows the general outline of that adopted by Yeatman (11, 12). The family Cyclopidae is divided into several genera. Species of eight of these, *Ectocyclops*, *Eucyclops*, *Macrocyclops*, *Mesocyclops*, *Orthocyclops*, *Paracyclops*, *Tropocyclops*, and *Cyclops*, are mentioned in this paper. Within the genus *Cyclops* as defined in this classification, there are several recognized groups of species. The species of the genus *Cyclops* mentioned in this paper may be placed in the following groups:

Microcyclops including *C.* (*M.*) *varicans rubellus* Lilljeborg
Acanthocyclops including *C.* (*A.*) *vernalis* Fischer
Acanthocyclops including *C.* (*A.*) *brevispinosus* Herrick
Acanthocyclops including *C.* (*A.*) *venustoides* Coker
Diacyclops including *C.* (*D.*) *bicuspidatus* Claus
Diacyclops including *C.* (*D.*) *bicuspidatus thomasi* Forbes
Diacyclops including *C.* (*D.*) *navus* Herrick
Cyclops s.s. including *C. scutifer* Sars
Cyclops s.s. including *C. strenuus* Fischer

These groups of species have been considered either subgenera or genera by different workers. Present opinion among North American authorities appears to favor subgeneric rank for these groups. However, the authors of this paper believe that the fifth foot structure and incomplete segmentation of the swimming feet of *Microcyclops* are of sufficient distinction to set this group off as a separate genus. This will be discussed more fully in future papers.

Price (9) has shown that *C. vernalis* Fischer as described by present taxonomic methods is in effect a species group consisting of at least seven reproductive isolates. One of these isolates fits the description of *C. brevispinosus* Herrick and should be referred to by that name. Tentatively, the others have been designated *C. vernalis* A, B, D, E, F, and G.

C. bicuspidatus thomasi Forbes is a common limnetic cyclopid of North American lakes often referred to as *C. bicuspidatus*, and occasionally as *C. thomasi*. It should not be confused with the Eurasian *C. bicuspidatus* Claus, which probably occurs occasionally in a few limited habitats in North America.

Results

During the course of the experimental infections, 23 species of cyclopids were exposed to coracidia. Large numbers of some of the more abundant species were used in the experiments, but only a few of some less-common species could be obtained. Nineteen species were exposed to coracidia of both *T. crassus* and *T. nodulosus*, three were exposed to *T. crassus* only, and one was exposed to *T. nodulosus* only. The number of individuals of each species exposed, the number infected, and the resultant percentage infection is shown in Table I for *T. crassus* and in Table II for *T. nodulosus*.

Cyclopids which were infected with one species of *Triaenophorus* were generally also infected with the other. Individuals of *Microcyclops varicans rubellus* were found to be infected most frequently with both species of *Triaenophorus*. Individuals of seven species of *Cyclops*, including *C. brevispinosus*, *C. bicuspidatus thomasi*, *C. navus*, and four reproductive isolates of *C. vernalis*, as well as *Mesocyclops edax*, were also infected with both parasites. *Macrocyclops albidus*, *M. fuscus*, and *Ectocyclops phaleratus* were infected with *T. crassus* but not with *T. nodulosus*, while *Eucyclops speratus* and two isolates of *Cyclops vernalis* were infected with *T. nodulosus* and not *T. crassus*. *Cyclops venustoides*, *Orthocyclops modestus*, *Tropocyclops prasinus*, and *Eucyclops agilis* were exposed to coracidia of both species without infection. *Cyclops scutifer* was exposed to *T. nodulosus* only and became infected, while *Mesocyclops leuckarti*, *Eucyclops prionophorus*, and *Paracyclops fimbriatus* were exposed to *T. crassus* coracidia only with negative results.

The procercoids which developed in the haemocoele of most species of infected cyclopids were similar and possessed typical cercomeres bearing the embryonic hooks. The procercoids found in *Eucyclops speratus* and *Ecto-*

TABLE I

The number of each species of cyclopid exposed to, and the number and percentage infected with, *Triaenophorus crassus* at Toronto and Heming Lake

Species	Toronto experiment			Heming experiment			Total			Rank
	Exposed	Infected	%	Exposed	Infected	%	Exposed	Infected	%	
Microcyclops varicans rubellus	39	35	89	13	8	62	54	43	80	1
Cyclops vernalis E	16	12	75	—	—	—	16	12	75	2
Cyclops navus	17	14	82	2	0	—	19	14	74	3
Cyclops vernalis G	46	22	48	—	—	—	46	22	48	4
Ectocyclops phaleratus	2	1	50	3	1*	33	5	2	40	5
Cyclops vernalis F	35	10	29	—	—	—	35	10	29	6
Cyclops bicuspidatus thomasi	50	11	22	184	55	30	234	66	28	7
Mesocyclops edax	61	16	26	45	0	—	106	16	14	8
Macrocyclops fuscus	33	3	9	6	0	—	39	3	8	9
Cyclops vernalis A	46	3	7	—	—	—	46	3	7	10
Cyclops brevispinosus	10	0	—	28	2	7	38	2	5	11
Macrocyclops albidus	24	1	4	6	0	—	30	1	3	12
Cyclops vernalis B	20	0	—	—	—	—	20	0	—	
Cyclops vernalis D	31	0	—	—	—	—	31	0	—	
Cyclops venustoides	14	0	—	—	—	—	14	0	—	
Mesocyclops leuckarti	15	0	—	—	—	—	15	0	—	
Eucyclops speratus	18	0	—	29	0	—	47	0	—	
Eucyclops agilis	14	0	—	25	0	—	39	0	—	
Eucyclops prionophorus	—	—	—	12	0	—	12	0	—	
Paracyclops fimbriatus	—	—	—	14	0	—	14	0	—	
Tropocyclops prasinus	—	—	—	22	0	—	22	0	—	
Orthocyclops modestus	—	—	—	13	0	—	13	0	—	

*Procercoids in these infected cyclopids were abnormal and lacked cercomeres.

cyclops phaleratus did not possess a cercomere and therefore are regarded as atypical. It is not known whether these would have infected the next intermediate host.

The results of the infection of several mass cultures are included in Tables I and II. The mass cultures were carried out to infect quantities of *C. bicuspidatus thomasi* and *C. brevispinosus* because of the relative ease with which fairly large numbers of infected cyclopids could be obtained. While the results may not be exact, they are comparable and were therefore included.

TABLE II

The number of each species of cyclopid exposed to, and the number and percentage infected with, *Triaenophorus nodulosus* at Toronto and Heming Lake

Species	Toronto experiment			Heming experiment			Total			Rank
	Exposed	Infected	%	Exposed	Infected	%	Exposed	Infected	%	
Microcyclops varicans rubellus	23	19	83	—	—	—	23	19	83	1
Cyclops vernalis E	22	16	70	—	—	—	22	16	70	2
Cyclops vernalis G	55	35	60	—	—	—	55	35	60	3
Cyclops bicuspidatus thomasi	77	23	30	123	53	44	200	76	38	4
Cyclops varus	36	11	31	—	—	—	36	11	31	5
Cyclops vernalis F	27	8	29	—	—	—	27	8	28	6
Cyclops brevispinosus	11	3	27	36	9	25	47	12	26	7
Cyclops vernalis B	19	3	16	—	—	—	19	3	16	8
Cyclops scutifer	13	2	15	—	—	—	13	2	15	9
Cyclops vernalis A	47	5	11	—	—	—	47	5	11	10
Mesocyclops edax	18	4	22	28	0	—	46	4	9	11
Cyclops vernalis D	55	2	4	—	—	—	55	2	4	12
Eucyclops speratus	—	—	—	46	1*	2	46	1	2	13
Eucyclops agilis	—	—	—	15	0	—	15	0	—	
Cyclops venustoides	10	0	—	—	—	—	10	0	—	
Macrocyclops albidus	13	0	—	2	0	—	15	0	—	
Macrocyclops fuscus	4	0	—	—	—	—	4	0	—	
Tropocyclops prasinus	—	—	—	10	0	—	10	0	—	
Orthocyclops modestus	—	—	—	12	0	—	12	0	—	
Ectocyclops phaleratus	—	—	—	3	0	—	3	0	—	

*Procercoids in these infected cyclopids were abnormal and lacked cercomeres.

A summary of the individual infections by developmental stages (Table III) indicates that adult *C. bicuspidatus thomasi* are more easily infected with both parasites than immatures (fourth and fifth copepodite stages). Although samples of other species are not large enough to permit individual examination, the pooled data show the same relationship. Adult male *C. bicuspidatus thomasi* appear to be more readily infected by both parasites than either

TABLE III

Rate of infection of immature and adult cyclopids with *Triaenophorus crassus* and *T. nodulosus* (number of cyclopids in parenthesis)

	iv copepodite, %	v copepodite, %	Adult male, %	Adult female, %	Total adult, %
T. crassus					
C. bicuspidatus thomasi (Toronto and Heming data)	20.0 (70)	18.0 (39)	64.0 (11)	59.0 (17)	58.8 (28)
All species combined (except *C. b. thomasi*) (Toronto data)	19.4 (62)	23.3 (60)	43.8 (89)	29.4 (177)	34.2 (266)
T. nodulosus					
C. bicuspidatus thomasi (Toronto and Heming data)	7.4 (27)	29.3 (17)	85.0 (12)	29.0 (21)	49.0 (33)
All species combined (except *C. b. thomasi*) (Toronto data)	29.6 (27)	53.3 (15)	43.7 (103)	25.8 (182)	32.3 (285)

females or immature forms, and the fifth copepodite stage appears to be less resistant to infection (at least by *T. nodulosus*) than the fourth. Large quantities of yolky masses in adult females masked the presence of procercoids and a few infected individuals may have been overlooked on examination. No such obstacle existed to observation of males or immature individuals.

In the experiments, more than 1 procercoid developed in many of the animals; up to 10 procercoids were counted in some cases. The average number of procercoids per host was determined for several species at Toronto and Heming Lake, and is recorded in Table IV. Specimens of *Microcyclops varicans rubellus* support fewer procercoids per infected animal than do the specimens of the other larger species to which they are compared. This is undoubtedly the result of the smaller space available in the haemocoele of this species in comparison with that in the other species. The higher average of procercoids present in the Toronto experiments probably indicates that more coracidia were available to the infected cyclopids compared with the number used at Heming Lake. This is confirmed by a comparison with infections of cyclopids from nature where the numbers of coracidia present in the vicinity of a cyclopid are probably still lower. It has been the senior author's experience at Heming Lake that infected specimens from plankton tows almost invariably contain but one procercoid. Miller (6) has published similar results for Lesser Slave Lake, Alberta.

TABLE IV

The average number of procercoids of *Triaenophorus crassus* and *T. nodulosus* per infected cyclopid in the Toronto and Heming Lake experiments

Species	*T. crassus*, procercoids per infected animal		*T. nodulosus*, procercoids per infected animal	
	Toronto	Heming	Toronto	Heming
Cyclops bicuspidatus thomasi	5.4	3.5	2.9	1.4
Cyclops navus	5.4	—	1.9	—
Mesocyclops edax	4.9	—	—	—
Cyclops vernalis G	4.5	—	2.4	—
Cyclops vernalis E	3.3	—	1.9	—
Microcyclops varicans rubellus	2.6	2.0	1.9	—

Procercoids do not seem to die in the body cavity of infected hosts, nor do moderate numbers of them seem to affect the longevity of the host. The junior author determined the average number of procercoids per animal after infection and 30 days later, for individuals of several species, and found that while some of the cyclopids had died, the average number of procercoids per animal remained about the same. Infected cyclopids containing up to 10 procercoids have been kept for periods up to a month and a half before the cyclopid died. This approximates the maximum life span of uninfected animals under similar conditions. In all cases the number of procercoids present at the time of death was the same as when the cyclopid was first infected. Similar results were obtained by Miller (6) in his experimental infection.

Procercoids have been killed in living cyclopids after the animals were held for a short time at high temperatures. This was accidentally discovered when infected *M. edax* were found on subsequent examination to have lost their procercoids. Further trials showed that exposure to a temperature of 35° C for 5 minutes was sufficient to cause the death and subsequent dissolution of the procercoids in the few specimens available for experimentation.

A few experiments were performed to determine the number of coracidia eaten by cyclopids. Michajlow (5) demonstrated that large numbers of coracidia were eaten by all the cyclopids and diaptomids which he examined. This experiment attempted to disclose whether cyclopids which did not become infected ate coracidia.

Only a small number of experiments were carried out since sufficient quantities of coracidia were not available for a full-scale investigation. The eggs from which the coracidia were hatched had been stored under refrigeration for over a month and did not hatch too readily. The infection rate of the surviving cyclopids was much below that of the other experiments, so the coracidia may also have been less viable than those hatched earlier in the season and used for the remainder of the infections.

Individuals of all species tested ate coracidia (Table V). Results of the experiments do not indicate whether interspecific differences in the number of coracidia eaten exist, or whether the differences in the number of coracidia eaten in different tests were due to availability of coracidia, number of cyclopids per culture, or any other possible causes.

In the test with *Mesocyclops edax* about a fifth of the coracidia remaining after the cyclopids were removed were dead and distorted. It appears that these coracidia were destroyed by the cyclopids. They were probably ingested and regurgitated or egested without complete digestion.

TABLE V

Number of coracidia of *Triaenophorus crassus* and *T. nodulosus* eaten after 8 hours and resultant infections in experiments carried out at Heming Lake, July 1959

Species	Description of cyclopids introduced	No. of coracidia			No. of *Cyclops*	
		Introduced	Eaten/ cyclopid	% introduced eaten	Alive week later	Infected
T. crassus						
C. b. thomasi	5 mature ♀	54	9.0	83.3	4	1
C. b. thomasi	5 mature ♀	51	8.0	78.4	4	0
C. b. thomasi	5 mature ♀	51	5.2	51.0	2	0
C. b. thomasi	5 mature ♀	52	7.2	69.2	1	0
C. b. thomasi	15 mature ♀	240	13.0	81.3	0	—
C. b. thomasi	10 mature ♀	104	7.8	75.0	8	0
C. b. thomasi	4 mature ♀	61	9.8	64.5	3	1
C. b. thomasi	10 mature ♂	70	6.6	94.3	2	0
C. b. thomasi	1 iv copepodite	53	20.0	37.7	1	0
M. varicans rubellus	8 mature ♀	65	7.1	87.6	6	0
M. varicans rubellus	8 mature ♀	69	8.1	95.3	6	0
C. brevispinosus	7 mature ♀	129	16.7	98.2	5	0
C. brevispinosus	1 iii copepodite	58	14.0	24.1	1	0
M. edax	6 mature ♀	69	5.5	47.8	5	0
M. edax	6 mature ♀	97	12.3	76.9	2	0
E. agilis	6 mature ♀	72	11.7	97.5	4	0
E. agilis	6 mature ♀	60	9.7	97.0	0	—
T. nodulosus						
C. b. thomasi	5 mature ♀*	51	10.2	100.0	5	0
C. b. thomasi	5 mature ♀*	52	10.4	100.0	5	1

*These cyclopids remained in the counting cells with the coracidia for 48 hours.

No marked differences were noted between the number of coracidia eaten by small and large species or between species classed as herbivorous and carnivorous. Both the smallest cyclopids, *Microcyclops varicans rubellus*, and the largest cyclopids tested, *Mesocyclops edax*, ate considerable numbers of coracidia. *Microcyclops varicans rubellus* and *Eucyclops agilis*, which are both considered to be herbivores, ate coracidia as did *Cyclops bicuspidatus thomasi*, *C. brevispinosus*, and *Mesocyclops edax*, which are considered to be carnivores.

If the number of procercoids which developed from these exposures to coracidia is normal, these experiments also indicate that large numbers of coracidia may have to be eaten for infection to occur. It is felt that some of the coracidia may have been only partially viable because the eggs from which they hatched had been held for abnormally long periods of time, so that the number actually required may be less than what is implied by these experiments.

Discussion

Nine species of cyclopids tested in these studies were capable of acting as hosts for both *Triaenophorus crassus* and *T. nodulosus*. Six others were infected upon occasion with either one or the other of the two parasites. These results confirm Michajlow's (5) conclusion that several species of cyclopids would serve as hosts for *T. nodulosus*, some being more suitable than others. In addition, it seems possible from our data to state that *T. crassus* and *T. nodulosus* react in a similar manner to their first intermediate hosts.

Under standard conditions, a measure of the susceptibility of a cyclopid species would be the proportion of individuals infected on exposure to either or both species of *Triaenophorus*. The experiments described in this paper indicate that some cyclopids are more susceptible to infection than others. From these results it appears that *Microcyclops varicans rubellus* is the most suitable host for *Triaenophorus*. Other suitable hosts are found within the subgenera *Acanthocyclops*, *Diacyclops*, and *Cyclops* proper of the genus *Cyclops*. Cyclopids in the genera *Mesocyclops*, *Macrocyclops*, *Eucyclops*, and *Ectocyclops*, and some species of the *Cyclops vernalis* group apparently are less favorable hosts, while those of the genera *Tropocyclops*, *Paracyclops*, and *Orthocyclops*, as well as *Cyclops venustoides*, are even less suitable as hosts.

Failure of some cyclopids to ingest coracidia might result if the coracidia were too small (or too large) to be eaten readily, or if they belonged to a class of organisms which was not generally chosen as food. Any physiological state which would curtail the cyclopid's food intake would also temporarily prevent ingestion of coracidia.

In the present experiment, both large (*Mesocyclops edax*) and small (*Microcyclops v. rubellus*) cyclopids ate coracidia (Table V). There was no apparent relationship between the number of coracidia eaten and the size of the cyclopids in our tests. Ingestion experiments, using various numbers of coracidia and more cyclopids in the small and large size range, are necessary to clarify this point.

Cyclopids have been classified by several authors, including Fryer (3), as either carnivorous or herbivorous in their food habits. In this experiment, both herbivorous and carnivorous species ate coracidia. This is substantiated by the observation that herbivorous species such as *Eucyclops agilis* could be reared on cultures rich in protozoans. These species are possibly better described as omnivores rather than herbivores. It is impossible to say that these forms will not eat coracidia simply because their normal food preference is for plant material.

The junior author has found that individual cyclopids may cease feeding for short periods of time during various periods in the life history. Feeding is stopped, for instance, before and during molts, during copulation, and possibly at other times as well. Other workers have found that certain stages of the life history of many cyclopids are quiescent non-feeding resting stages (Elgmork (2)). If the individuals in our experiments were in any of these non-feeding phases for the short while coracidia were present, they would not ingest coracidia. Our results do not indicate how much this factor may have affected the ingestion of coracidia and the subsequent infection data, but it may be responsible for the differences in infection between developmental stages of cyclopids, especially *Cyclops bicuspidatus thomasi*. Cole (1) reports a resting stage in the fourth copepodite stage of this cyclopid. Oil droplets in the body cavity and apparently empty gut cavities, which characterize this condition, were noted in some of the fourth copepodite, *C. b. thomasi*, examined at Heming Lake. While it is impossible from the data to state the duration of this condition in our fourth copepodite animals, it probably influenced the results of the experimental infections.

Once coracidia are ingested some may be digested. Michajlow (5) found that coracidia were digested by many of the species of cyclopids which he tested. In our experiments there was no direct evidence that digestion of coracidia took place. However, as noted earlier, some destroyed coracidia which may have been partially digested were found after they had been introduced to a culture of *Mesocyclops edax*.

The development of the procercoid in the haemocoele does not always proceed successfully. In some cases aberrant procercoids were found. It is possible that others may have developed, died, and disappeared before the cyclopids were examined.

Since several species can serve at least experimenatlly as hosts for *T. crassus* and *T. nodulosus*, an attempt will be made to evaluate their potentialities as hosts in nature. The suitability of a species as a host depends not only on its susceptibility to infection, but also on its presence in areas where coracidia and the next host in the life cycle are found. These factors may be conveniently considered in terms of geographic range, abundance, and habitat preference. Data on the known range, habitat, and local abundance were collected from several sources, including Yeatman (11, 12) and Pennak (8), and were combined with our infection results to obtain a measure of their probable importance as natural hosts (Table VI).

TABLE VI

The importance of experimentally infected species as natural hosts of *Triaenophorus crassus* and *T. nodulosus*

Species	Habitat and habits	Relative abundance	Susceptibility	Importance as host
M. varicans rubellus	A pond species, littoral and non-planktonic, widespread	Never found by the authors in quantity	Excellent	Unknown, may be important
C. vernalis E and G	Pond species, non-planktonic, local	Probably rare	Very good	Not well fitted
C. navus	Pond, littoral species, widespread	Uncommon	Moderate	Not well fitted
C. vernalis F	Littoral, non-planktonic, permanent ponds, lakes. Local	Probably rare	Moderate	Not well fitted
C. scutifer	Limnetic, in cold northern waters. Widespread	Common to abundant	Moderate to poor	Potentially important
C. brevispinosus	Limnetic, occurring in shallows as well. Widespread	Common to abundant	Moderate to poor	Possibly important
C. bicuspidatus thomasi	Limnetic, occurring in shallows as well. Widespread	Abundant	Moderate	The most important
C. vernalis A, B, D	Littoral, in permanent and temporary water. Local	Unknown	Poor	Not important
M. edax	Limnetic, widespread	Common	Poor	Probably not important
Macrocyclops sp.	Littoral and in pools. Widespread	Common	Poor	Not important
Eucyclops sp.	Littoral, widespread	Abundant	Developed aberrant procercoids	Not important
Ectocyclops phaleratus	In vegetation, widespread	Rare	Developed aberrant procercoids	Not important

Of the most completely susceptible cyclopids apparently only *Microcyclops varicans rubellus* could be more than locally important as a vector. Of the moderately susceptible species, *Cyclops scutifer*, *C. bicuspidatus thomasi*, and *C. brevispinosus* are probably the only ones widely enough distributed and abundant enough to act as first intermediate hosts of *Triaenophorus crassus* and *T. nodulosus* throughout the range of these species. The majority of naturally occurring infections at Heming Lake, which lacks *C. scutifer*, occur in *C. bicuspidatus thomasi* although a few have been recorded from *C. brevispinosus*. None of the lightly infected species are likely to act as first intermediate hosts in nature, except locally, because so few of them become infected. Of these, *Mesocyclops edax* is potentially the best suited as a host because of abundance and widespread range.

Summary

(1) Nineteen species of cyclopids were exposed to coracidia of *Triaenophorus crassus* and *T. nodulosus*. Three were exposed to *T. crassus* only and one to *T. nodulosus* only. Procercoids of both parasites developed in nine species, including *Microcyclops varicans rubellus*, four reproductive isolates of the *C. vernalis* group, *C. brevispinosus*, *C. bicuspidatus thomasi*, *C. navus*, and *Mesocyclops edax*. *Macrocyclops albidus*, *M. fuscus*, and *Ectocyclops phaleratus* were infected with *T. crassus* and not *T. nodulosus*, while *Eucyclops speratus* and two isolates of *C. vernalis* were infected with *T. nodulosus*. *C. scutifer*, which was not exposed to *T. crassus*, was infected with *T. nodulosus*. *Eucyclops agilis*, *E. prionophorus*, *Cyclops venustoides*, *Tropocyclops prasinus*, *Orthocyclops modestus*, *Paracyclops fimbriatus*, and *Mesocyclops leuckarti* were not infected.

(2) Differences in the proportion of cyclopids infected were noted between animals of different degrees of maturity and between sexes.

(3) Most experimental infections were heavier than natural ones and the average number of procercoids per infected animal varied directly as the size of the species involved.

(4) Procercoids live throughout the life of the infected cyclopid and do not seem to shorten the host's life appreciably. Exposure to high temperatures of the order of 35° C for a short time was found to cause the death and disappearance of procercoids in living *M. edax*.

(5) Large size and herbivorous food habits do not seem to prevent the ingestion of coracidia by the species of cyclopids examined and apparently large numbers of coracidia must be eaten for the development of procercoids in *C. bicuspidatus thomasi*.

(6) A review of the distribution, habitat, and infective properties of the species examined indicates that *C. bicuspidatus thomasi* and probably *C. brevispinosus* and *C. scutifer* are potentially the most important natural intermediate hosts of the two species of *Triaenophorus* examined. There is no reason why *Microcyclops varicans rubellus* should not also be an important natural host.

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References

1. Cole, G. A. Notes on copepod encystment. Ecology, **34** (1), 208–211 (1953).
2. Elgmork, K. Seasonal occurrence of *Cyclops strenuus strenuus*. Folia Limnologica Scand. **11**, 1–196 (1959).
3. Fryer, G. The food of some freshwater cyclopoid copepods and its ecological significance. J. Animal Ecol. **26**, 263–286 (1957).
4. Michajlow, W. *Triaenophorus crassus* Forel, et son développment. Ann. parasitol. humaine et comparée **10** (3), 257–270 (1932).
5. Michajlow, W. Les adaptations graduelles des copépodes comme premiers hôtes intermédiares de *Triaenophorus*. Ann parasitol. humaine et comparée, **10** (4), 334–344 (1932).
6. Miller, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. II. The eggs, coracidia, and life in the first intermediate host of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas). Can. J. Research, D, **21**, 284–291 (1943).

7. MILLER, R. B. A review of the *Triaenophorus* problem in Canadian lakes. Bull. Fisheries Research Board Can. No. 95, 1–42 (1952).
8. PENNAK, R. W. The fresh-water invertebrates of the United States. Ronald Press, New York. 1953. pp. 383–409.
9. PRICE, J. L. Cryptic speciation in the *vernalis* group of Cyclopidae. Can. J. Zool. **36**, 285–303 (1958).
10. ROSEN, F. Recherches sur le développment des cestodes. I. Le cycle évolutif des Bothriocephales. Bull. soc. neuchâtel. sci. nat. **43**, 247–300 (1919). (In translation.)
11. YEATMAN, H. C. American cyclopoid copepods of the *viridis–vernalis* group (including a description of *Cyclops carolinianus* n. sp.). Am. Midland Naturalist, **32** (1), 1–90 (1944).
12. YEATMAN, H. C. Cyclopoida. *In* Ward and Whipple's Freshwater biology. 2nd ed. John Wiley, New York. 1959. pp. 785–813.

NOTES ON SOME POLYCHAETA FROM THE WEST COAST OF MEXICO, PANAMA, AND CALIFORNIA[1]

E. Berkeley and C. Berkeley

Abstract

Notes on 13 species of Polychaeta selected from collections made on, or near, the coasts of California and Mexico during 1958 and 1959 are recorded. Of these one (*Eurythoe complanata* var. *mexicana*) is a new variety, three (*Paraeurythoe americana*, *Halla parthenopeia*, *Asychis amphiglypta*) are new records for the eastern Pacific, three (*Perinereis obfuscata*, *Nereis oligohalina*, *Polyophthalmus pictus*) involve new records of swarming forms, and two (*Glycera robusta*, *Aphrodite falcifera*) are extensions of distribution. The remainder deal with species having miscellaneous features of some other interest.

Introduction

We reported in 1958 (5) on a collection of Polychaeta from the northeast Pacific south of latitude 32° made by Mr. H. L. Klawe of the Inter-American Tuna Commission in the years 1955, 1956, and 1957. During 1958 and 1959 we received further material from the same source, mainly collected in Mexican waters, and during 1959 Mr. H. Arai of the University of California, Los Angeles, has also sent us small collections from the same region, whilst quite recently we have received specimens of a very interesting maldanid from Mr. W. A. Newman, of the University of California, Berkeley. Many of the species in these collections have already been recorded from the region and are fully described elsewhere. The present paper deals only with those items in them which have noteworthy features. The individual collectors are indicated in the text by the initials H. L. K., H. A., and W. A. N., respectively.

Aphroditidae

Aphrodite falcifera Hartman. Hartman (14)

A single example of this species, from San Diego, California (H.L.K.), is 32 mm long and 14 mm wide as preserved, considerably longer than the type, and only previously known, specimen. This was 22 mm long and was recorded from Carros Island, Mexico. Its distribution is now extended northwards.

Polynoidae

Chaetacanthus magnificus (Grube). Seidler (28)

Two specimens, both about 30 mm long and 5 mm wide, taken in dead coral at Zihnatanejo, Guerro Province, Mexico (H.L.K.). Seidler (28) and Hartman (14) regard *Polynoe branchiata* Treadwell (29) as synonymous with *Chaetacanthus magnificus* (Grube) and Hartman adds *Lepidonotus pilosus* Treadwell (31) to the synonymy. The present specimens have been identified

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from a joint consideration of Seidler's and Treadwell's descriptions. The species seems to be somewhat variable. The specimen from Espiritu Santo Island, Mexico, recorded by us (2) as *Lepidonotus pilosus* Treadwell, for instance, having elytra with much less heavy fringes and smaller tubercles than the present examples, so that the dorsum has a much smoother appearance.

Originally described from the West Indies this species has been recorded previously from the eastern Pacific from Ecuador to the Gulf of California (14).

Amphinomidae

? Paraeurythoe americana Hartman. Hartman (18)

A single specimen taken at night at Balboa, Panama (H.L.K.), is a ripe female distended with eggs. It is considerably contracted as preserved, measuring 85 mm long and 5 mm wide and thus small by comparison with the dimensions given by Hartman (18). In this respect and in that, owing to the small size and retraction of the prostomium, we have been unable to determine the presence or absence of a caruncle with certainty, some doubt attaches to our identification, but agreement in all other particulars is close.

P. americana is recorded only from the Gulf of Mexico, but Hartman (18) suggests synonymy with *Eurythoe dubia* Monro (21), which is recorded from the same locality as the present specimen. Our observations lend probability to this synonymy.

Eurythoe complanata (Pallas) var. *mexicana* var. n. Hartman (15) (stem-species)

An anterior portion, about 40 mm long and 5 mm wide, consisting of some 30 segments, and an anal portion, about 32 mm long and 3 mm wide, from San Carlos Bay, Gulf of California (H.A.), agree with this widely distributed species except in one respect. In both specimens most of the notosetae in all the parapodia closely resemble the abnormal form described by us (2, Fig. 1) from a specimen taken at Espiritu Santo Island, Gulf of California, the only difference being that the tips are less expanded. In some parapodia all the smooth notosetae are displaced by these, but a few of the large serrate ones always remain. That they are not worn or broken shafts of normal setae is clear from the fact that many which have not yet penetrated to the exterior can be seen in the notopodial lobe. When we observed these setae first, we regarded their presence as an individual idiosyncrasy of the specimen in which they were found, but the present second case of their occurrence suggests that it has at least varietal significance.

Glycera robusta Ehlers. Ehlers (7), Berkeley and Berkeley (4)

A fine specimen, 600 mm long as preserved, from San Carlos Bay, Gulf of California (H.A.). This appears to be the most southerly station at which this species has been collected on the east coast of the Pacific. It is common farther north. Okuda (24) records it from Nakanoshima, south Japan, in approximately the same latitude as that at which the present specimen was taken.

Nereidae

Nereis (*Ceratonereis*) *tentaculata* Kinberg. Hartman (15) (synonymy); Ehlers (8), as *C. mirabilis*; Treadwell (30), as *C. singularis*

Two specimens of the atokous stage of this species from dead coral, from Zihnatanejo, Mexico, and several heteronereids from Le Paz Harbour, Gulf of California (H.L.K.). All the heteronereids except one are males. They are all more or less broken, but specimens can be reconstructed and show three distinct body regions. This is in accordance with Treadwell's description (30) of the heteronereids of *Ceratonereis singularis*, a synonym of the present species. Treadwell finds that the change to the epitokous parapodium occurs after the 16th somite and that the terminal region consists of about 25 somites. In our specimens the change occurs (in males) at the 22nd or 23rd somite and the number of somites in the posterior region is very variable, as many as a hundred or so being present in some individuals. The species is widely distributed in southern latitudes.

Perinereis obfuscata Grube. Monro (20), Rioja (25)

Two heteronereids of this species, one of each sex, from Zihnatanejo, Mexico (H.L.K.). Rioja's description (25) of atokes from Acapulco, Mexico, corresponds closely with the present specimens except in so far as they are modified by heteronereid characters. Monro (20) describes epitokes of both sexes and with these also our specimens are in complete agreement. The male has 14 setigers anterior to the first epitokous parapodium, the female 18.

Nereis oligohalina Rioja. Rioja (26)

Several male heteronereids and a few females from Le Paz Harbour, Gulf of California (H.L.K.). We recorded in our 1958 paper (5) that the former has 17 setigers anterior to the first epitokous parapodium. To this may now be added that there are 22 such setigers in the female.

Eunicidae

Halla parthenopeia (Delle Chiaje). Fauvel (10), Okuda (23), Ehlers (7), as *Cirrobranchia*

A single specimen 100 mm long, 8 mm wide, collected by J. Wintersteen (submitted to us by H.A.), at San Luis Bay, Gulf of California, intertidal, agrees closely with Fauvel's and Ehler's descriptions except in that we have not been able to find the hispid capillary setae described by the former, and the latter omits mention of the bidentate hooded crotchets which Fauvel describes and are present in our specimen. The three tentacles and the first segment are much contracted and withdrawn into the second segment, so that the characteristic tentacular depression can be seen only with difficulty. The body color is a rather dark brown as preserved.

Halla parthenopeia is one of the largest errant polychaetes known, attaining a length of 90 cm, so that the present specimen must be regarded as a very young individual. It has not been recorded previously from the eastern Pacific, but is fairly common in Japan (23).

Spionidae

Scololepis indica Fauvel. Fauvel (12 and 13), Berkeley and Berkeley (3)

A single individual incomplete posteriorly from San Carlos Bay, Gulf of California, intertidal (H.A.), agrees exactly with Fauvel's descriptions. We recorded specimens from Mission Bay, California, in 1941 (3), since which the only notification of its occurrence seems to be that of Day ((6), as *Scolecolepis*) from South Africa. In the example from Mission Bay we found tridentate-hooded crotchets in some parapodia and similar instances are recorded by Day (6). These are not mentioned in the original descriptions and we have found none in the present specimens.

Maldanidae

Clymene (*Euclymene*) *grossa* Baird var. *newporti* Berkeley. Berkeley and Berkeley (3), Ehlers (9) (stem-species)

A single example from Point Loma, California, intertidal (H.L.K.), is noteworthy by reason of its size. It is 200 mm long and 8 mm wide as preserved, considerably larger than any previous record of either the stem-species or of the variety. Of the former the largest known is 125 mm long and 6 mm wide and, of the latter, 70 mm long by 4 mm wide.

Asychis amphiglypta (Ehlers). Ehlers (8), as Maldane; Arwidsson (1); Monro (22)

Many anterior and posterior halves of specimens dredged in 3 to 6 meters in South San Francisco Bay, California (W.A.N.), agree closely with this species as described fully both by Ehlers and Arwidsson. Since all the specimens, except one or two very small ones, are broken we cannot determine a maximum length. The greatest width of the widest fragment is 3 mm as preserved. The species is characterized by having the cephalic border divided by a deep notch on each side into two lobes the edges of which are quite entire, but may be more or less undulate. The nuchal grooves are short and bent into a crescent anteriorly, but the posterior end of the groove is frequently obscure and the grooves then appear as transverse crescents. There is no collar on the first setiger. There is only one preanal segment which is frequently not well defined. The margin of the anal plate of the pygidium is extended and divided into two lobes, the ventral one forming a pocket over the plate, the dorsal extending beyond the anus. The latter lobe has no trace of border filaments such as Monro (22) found in some specimens of the present species and which characterize several other members of the genus.

The species has been taken previously only from subantarctic stations, so that its distribution is considerably extended by the present record. *Asychis maculata* (Kinberg), as briefly described both by Kinberg (19) and Hartman (17), seems to be near *A. amphiglypta*. It is recorded from India.

Opheliidae

Polyophthalmus pictus (Dujardin). Fauvel (11)

Many specimens taken swarming at a light at Zihnatanejo, Mexico (H.L.K.), chiefly males. This widespread species has been recorded in the atokous stage from Acapulco, Mexico (25), from the Gulf of California (27, 3), and from California (14, 16). The epitokous stages are known from the Mediterranean region (11), but have not been recorded previously from the Pacific. They differ from the atokous form in that the setae are considerably elongated in the last five or six setigers and the nephridial openings are dilated.

References

1. Arwidsson, I. Die Maldaniden. Wiss. Ergeb. Schwed. Südpolar Expedition, 1901–1903, **6**, Lief. 6, 1–44 (1911).
2. Berkeley, E. and Berkeley, C. On a collection of Polychaeta, chiefly from the west coast of Mexico. Ann. and Mag. Nat. Hist. Ser. 11 (3), 321–346 (1939).
3. Berkeley, E. and Berkeley, C. On a collection of Polychaeta from southern California. Bull. Southern Calif. Acad. Sci. **40** (1), 16–60 (1941).
4. Berkeley, E. and Berkeley, C. Polychaeta Errantia. Fisheries Research Board Can., Can. Pacific Fauna, No. 9*b* (1), 1–100 (1948).
5. Berkeley, E. and Berkeley, C. Some notes on a collection of Polychaeta from the northeast Pacific south of latitude 32° N. Can. J. Zool. **36**, 399–407 (1958).
6. Day, J. H. The polychaete fauna of South Africa. Pt. 1. The intertidal and estuarine Polychaeta of Natal and Mosambique. Ann. Natal Museum, **12**, Pt. 1, 1–67 (1951).
7. Ehlers, E. Die Borstenwürmer. Annelida Chaetopoda. Leipzig, Wilhelm Engelmann, 1–748 (1864–1868).
8. Ehlers, E. Polychaeten. Hamburger Magalhaenische Sammelreise, 1–140 (1897).
9. Ehlers, E. Die Polychaeten des Magellanischen und Chilenischen Strandes. Berlin, Weidmannsche Buchhandlung, 1–224 (1901).
10. Fauvel, P. Polychètes errantes. Faune de France, No. 5. 1923.
11. Fauvel, P. Polychètes sédentaires. Faune de France, No. 16. 1927.
12. Fauvel, P. Annélides Polychètes nouvelles de l'Inde. Bull. muséum hist. nat. No. 1, 1–7 (1928).
13. Fauvel, P. Annelida Polychaeta of the Indian Museum, Calcutta. Mem. Indian Museum Calcutta, **12**, 1–262 (1932).
14. Hartman, O. Polychaetous annelids. Pt. 1. Aphroditidae to Pisionidae. Allan Hancock Pacific Expeditions, **7**, 1–352 (1939).
15. Hartman, O. Polychaetous annelids. Pt. 2. Chrysopetalidae to Goniadidae. Allan Hancock Pacific Expeditions, **7**, 173–258 (1940).
16. Hartman, O. Polychaetous annelids from California. Allan Hancock Pacific Expeditions, **10**, 239–290 (1944).
17. Hartman, O. The marine annelids erected by Kinberg. Arkiv Zool. **42A** (1), 1–137 (1948).
18. Hartman, O. The littoral marine annelids of the Gulf of Mexico. Publs. Inst. Marine Sci. **2** (1), 7–124 (1951).
19. Kinberg, J. G. H. Annulata Nova. K. Vetenskaps-Akademiens, No. 9, 337–357 (1866).
20. Monro, C. C. A. Polychaeta, Oligochaeta, Echiuroidea and Sipunculoidae. Great Barrier Reef Expedition 1928–29, 1–37 (1931).
21. Monro, C. C. A. The Polychaeta Errantia collected by Dr. C. Crossland at Colón in the Panama region and the Galapagos Islands during the expedition of the S. Y. St. George. Proc. Zool. Soc. London, Pt. 1, 1–96 (1933).
22. Monro, C. C. A. Polychaete worms. Discovery Reports, **12**, 59–198 (1936).
23. Okuda, S. Some polychaete annelids used as bait in the Inland Sea. Annotationes Zool. Japon. **14**, 243–248 (1933).
24. Okuda, S. Annelida Polychaeta in Onagawa Bay and its vicinity. 2. Polychaeta Errantia with some addenda of Polychaeta Sedentaria. Sci. Repts. Tôhoku Imp. Univ. **14**, (2 and 3), 219–244 (1939).
25. Rioja, E. Estudios anelidologicos. 3. Datos para el concocimento de la fauna de poliquetos de las costas del Pacifico de Mexico. Anales inst. biol. Univ. nacl. Méx. **12**, 669–746 (1941).
26. Rioja, E. Estudios anelidologicos. 15. Nereidos de agua salobre de los esteros del litoral del Golfo de Mexico. Anales inst. biol. Univ. nacl. Méx. **17**, 205–214 (1946).

27. RIOJA, E. Estudios anelidologicos. 17. Contribucion al concimento de los anelidos poliquetos de Baja California y Mar de Cortes. Anales inst. biol. Univ. nacl. Méx. **18**, 198–224 (1947).
28. SEIDLER, H. J. Beiträge zur Kenntnis der Polynoiden. I. Arch. Naturgeschichte, **89**, Abt. A, Heft 11, 1–217 (1923).
29. TREADWELL, A. L. The polychaetous annelids of Porto Rico. Bull. U.S. Fish Comm. **20**, Pt. 2, 181–210 (1902).
30. TREADWELL, A. L. New species of polychaetous annelids in the collections of the American Museum of Natural History, from Porto Rico, Florida, Lower California and British Somaliland. Am. Museum Novitates, No. 392, 1–13 (1929).
31. TREADWELL, A. L. Polychaetous annelids from the west coast of Lower California, the Gulf of California and Clarion Island. Zoologica, **22**, 139–160 (1937).

VARIATION IN NUMBER AND ASYMMETRY IN BRANCHIOSTEGAL RAYS IN THE FAMILY ESOCIDAE[1]

E. J. CROSSMAN

Abstract

In teleost fishes which have high numbers of meristic parts there is great variability. Within the family Esocidae this variability is apparent in the branchiostegal rays. Within populations there often exists up to 23 combinations of numbers of these rays on the epihyoid and ceratohyoid bones on each side of single individuals. There is considerable bilateral asymmetry in both number and arrangement of these rays. Counts of the number of branchiostegal rays on each hyoid segment may prove more useful as distinguishing charactertistics than total counts now in use.

The number of branchiostegal rays has long been used to separate species of the genus *Esox*. Most often counts of the rays of only one side are given and we are left to assume which side. If these counts are to be used as distinguishing characters and in systematic comparisons of individuals from various populations it is imperative to know the intrapopulation variation. It is only with knowledge of the variability within a single population that the significance of the variation over the whole range of the animal can be truly determined.

Within a single population of any species of this family, number and arrangement of these rays are variable. In fishes with low numbers of branchiostegal rays the number is often constant for whole families, in the esocids and other fishes with a high number the variability is great and the number and arrangement on the two sides of a single individual often differ and exhibit bilateral asymmetry.

In the family Esocidae the paired hyoid bones each consist of two somewhat triangular-shaped halves, the epihyal and the ceratohyal, joined at their bases by a cartilaginous "hinge". The bases of the branchiostegal rays are attached to these two portions in such a way as to be divisible into two sections, anterior and posterior. Those rays connected to the anterior half of the hyoid bone, "anterior rays", originate on the dorsal surface or, rarely, on the medial edge of the ceratohyal. Those rays arising from the posterior portion of the hyoid bone, "posterior rays", originate on the ventral surface of the epihyal (see Fig. 1). In this way two groups of rays, the anterior and posterior, are readily separated. It is in the number and arrangement of the branchiostegal rays, arising from each of these two portions of the hyoid bone on each side of an individual, that the variation and asymmetry are evident.

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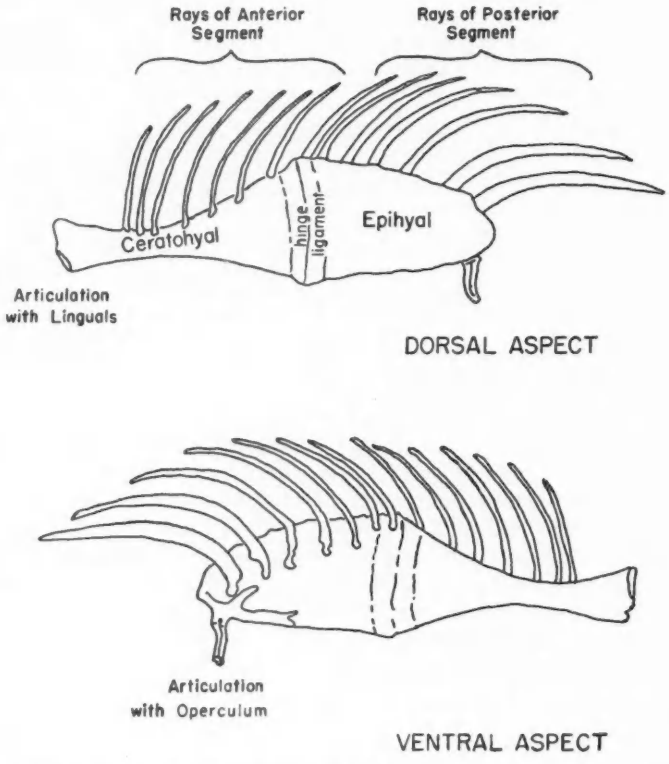

FIG. 1. Left hyoid bone of *Esox lucius* showing the usual arrangement of branchiostegal rays.

Materials

For this analysis of intrapopulation variation in number and arrangement of branchiostegal rays in the family Esocidae the following specimens were used:

(1) *Esox lucius* Linnaeus, northern pike: 150 individuals from Heming Lake, Manitoba. Supplied by Dr. G. H. Lawler, Fisheries Research Board of Canada.

(2) *Esox masquinongy* Mitchell, muskellunge: 70 individuals from Nogies Creek, Ontario. This sample consists of 35 specimens from each of two year classes from a single population. R.O.M. Nos. 19845* and 19045.

(3) *Esox niger* LeSueur, chain pickerel: 32 individuals from Stearns Pond, Massachusetts, supplied by Dr. E. C. Raney, Cornell University.

(4) *Esox americanus* Gmelin, redfin pickerel: 64 individuals from a single location near Lumberton, North Carolina. R.O.M. No. 20229.

*Cat. No. Fish Collection, Royal Ontario Museum.

(5) *Esox vermiculatus* LeSueur, grass pickerel: 20 specimens from one population on Point Pelee, Ontario. R.O.M. No. 17865.

(6) *Esox reicherti* Dybowski, Amur pike: four individuals, R.O.M. No. 18919, U.S.N.M. No. 77009,* two from U.S.N.M. No. 105190, all from Amur River Basin, U.S.S.R.

Since the subspecific relationship of the grass pickerel and the redfin pickerel has never actually been demonstrated, the nomenclature here follows Scott (1958 (2)) designating them as full species.

Branchiostegal ray counts given in the discussion will be by numbers (i.e. 7+9:7+9) which refer to the number of rays in the right anterior segment plus the number in the right posterior segment followed by similar counts for the left side.

TABLE I

Frequencies of the number of branchiostegal ray counts in species of the family Esocidae

Total two sides

Number of rays	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	N	$\bar{x}$
E. masquinongy													2	4	10	28	22	2	2	70	35.1
E. lucius							1	4	16	24	82	18	5							150	29.7
(*E. reicherti*)							3	1												4	—
E. niger								2	3	16	5	4	1	1						32	30.4
E. americanus			4	0	8	10	16	10	13	2	0	1								64	26.1
E. vermiculatus	1	0	0	3	5	8	1	2												20	24.4

Total right side only

Number of rays	9	10	11	12	13	14	15	16	17	18	19	20	N	$\bar{x}$
E. masquinongy								4	34	31	1		70	17.4
E. lucius					3	31	104	12					150	14.8
(*E. reicherti*)					3	1							4	—
E. niger						5	18	8	1				32	15.2
E. americanus			1	16	26	17	3	1					64	13.1
E. vermiculatus	1	0	7	10	2								20	11.6

Total left side only

Number of rays	9	10	11	12	13	14	15	16	17	18	19	20	N	$\bar{x}$
E. masquinongy								5	17	43	4	1	70	17.7
E. lucius					5	28	98	19					150	14.9
(*E. reicherti*)					4								4	—
E. niger						2	22	6	2				32	15.3
E. americanus			1	16	27	19	1						64	13.0
E. vermiculatus		1	4	12	3								20	11.8

Intrapopulation Variation in Number of Branchiostegal Rays

From the standpoint of comparison with total variation and to provide a background for the discussion of asymmetry Table I gives a summary of variation, within single populations of each species, in the number of branchiostegal rays. The total number of rays for both sides and right- and left-side counts are given.

There is considerable overlap in the total counts, which range from 20 to 27 in *E. vermiculatus*, the smallest species, to 32 to 38 in *E. masquinongy*, the largest member of the family. The species are arranged in Table I in approximate order of size and the counts descend in a somewhat regular fashion. While these are only isolated counts from widely separated areas it would be interesting to speculate on the reasons for the lack of alignment in this regard of *E. niger* which extends beyond *E. lucius*.

*Cat. No. Fish Collection, United States National Museum.

Average total number of rays readily separates *E. masquinongy* from all other members of the family. Total ray count alone is not sufficient to separate the other members since *E. niger* and *E. lucius* show similar mean total counts and the differences between the pickerels (*E. niger*, *E. americanus*, and *E. vermiculatus*) are insufficient, with such wide variability.

There is a suggestion from the four counts made that *E. reicherti* lies in the area of *E. americanus* in total and side counts.

When, however, branchiostegal ray counts are separated into hyoid segment counts (anterior and posterior) and single side counts are compared, there are striking differences. The modal combination of anterior and posterior counts and the next most frequent combinations in symmetrical individuals are shown below:

Species	Modes of single side branchiostegal ray counts (ant. + post.)
E. masquinongy	8+10 (8+9)
E. lucius	7+ 8 (6+8)
(*E. reicherti*)	5+ 8 (6+7)
E. niger	6+ 9 (7+9)
E. americanus	6+ 8 (5+8)
E. vermiculatus	5+ 7 (4+7)

The counts given above are the commonest symmetrical counts found in each species. There is however considerable bilateral asymmetry involved in the number and arrangement of these rays in the family Esocidae.

Variation and Bilateral Asymmetry in Number of Branchiostegal Rays

Hubbs and Hubbs (1945 (1)), investigating bilateral asymmetry and bilateral variation in fishes, discussed, along with the well-known observations in flatfishes, less-known occurrences in other body parts of many fishes. In their discussion they mention the bilateral asymmetry in branchiostegal rays in Salmonidae and Esocidae in particular. They include detailed counts for 442 *E. vermiculatus* from various localities in Michigan. They give no details on the variability or degree of asymmetry found within populations. They did, however, make hyoid segment ray counts. They expressed asymmetry as greater numbers of rays on one side and as sidedness in overlap of branchiostegal membranes.

Hyoid segment counts make it obvious, however, that even in individuals which have equal numbers of rays on each side (symmetrical to Hubbs and Hubbs) there is further asymmetry in the number of rays arising from each hyoid segment on each side. This is discussed below by species in regard to the percentage of asymmetry and variation in number and arrangement of hyoid segment rays. Complete data on actual counts for each species are given in Appendix Tables I and II.

Symmetrical, as used here, is taken to mean the same *number and arrangement* of anterior and posterior rays on each side.

Esox masquinongy

In the sample of 70 individuals from a single population, 53 (or 75.8%) were bilaterally asymmetrical in number and/or arrangement of rays. Only 17 (or 24.2%) were symmetrical. An additional 6 (or 8.5%) were symmetrical in number of anterior and posterior rays but not necessarily in arrangement. The symmetrical individuals showed five combinations of anterior and posterior ray counts (see Appendix Table I) ranging from 7+9 to 9+10 with a mode at 8+10. The total ray count (both sides), in ranging from 32 to 38, exhibited 23 combinations of anterior and posterior counts from 7+9:7+9 to 9+10:9+10. Eighteen of these combinations were asymmetrical. The rays on the right side varied in number from 16 to 21 in 6 combinations from 7+9 to 9+10, while those on the left varied from 16 to 20 in 10 combinations from 6+10 to 10+10.

There is variation from year class to year class within one population. In this population of muskellunge one year class had 33 to 38 rays (total), 16 to 19 (right side), and 16 to 20 (left side), while similar counts for the succeeding year class were 32 to 36, 16 to 18, and 16 to 18. The specimens used were hatchery-reared and this difference may be due to differences in artificial conditions during rearing in the two successive years.

Esox lucius

Asymmetrical individuals comprised 55% (83 in 150) of the sample of pike. There were 67 (45%) bilaterally symmetrical individuals and an additional 26 (17%) were symmetrical in number only. The symmetrical individuals showed seven combinations of ray counts from 6+7 to 8+8 with a mode at 7+8. The total ray count, ranging from 26 to 32, exhibited 30 combinations of counts from 5+8:7+7 to 9+7:7+9, with the same mode as the symmetrical individuals. Twenty-three of these combinations were asymmetrical. The right side varied from 13 to 16 rays in 11 combinations from 5+8 to 9+7 and over the same range the left side showed 9 combinations from 5+8 to 8+8.

Esox niger

In the sample of 32 chain pickerel 15 individuals (or 47%) were asymmetrical, 17 (or 53%) were symmetrical, and an additional 3 (or 9%) were symmetrical in number of rays only. The 17 symmetrical individuals showed three combinations of ray counts: 6+8, 6+9, and 7+9 with a mode at 6+9. Total ray count (both sides) ranged from 28 to 34 and was composed of 14 combinations, 11 of which were asymmetrical, ranging from 5+9:5+9 to 8+9: 7+10 with the same mode as above, 6+9:6+9. Right-side counts ranging from 14 to 17 showed eight combinations from 5+9 to 8+9 and over the same range the left side exhibited six combinations from 5+9 to 7+10.

Esox americanus

The single population sample of redfin pickerel consisting of 64 specimens had 42 individuals (65%) which were asymmetrical in number and/or arrangement or rays, 22 (34%) symmetrical, and an additional 3 (5%) symmetrical in number only. The 22 symmetrical fish showed six combinations of counts

from 4+7 to 6+8. The total sample, in which the two-side ray count ranged from 22 to 31, showed 28 combinations of anterior and posterior counts from 4+7:4+7 to 8+8:7+8, of which 22 were asymmetrical. Right-side counts from 11 to 16 occurred in 10 combinations from 4+7 to 8+8 and left side numbers ranging from 11 to 15 showed the same number of combinations but consisted of combinations from 4+7 to 7+8.

Esox vermiculatus

This species, in a sample of 20 from one location, had 8 (45%) asymmetrical individuals. Asymmetry in this case was in number alone. There was no asymmetry of arrangement in this sample or other samples of this species examined. The remaining 11 (or 55%) were symmetrical. The ray counts of the symmetrical individuals were in three combinations: 4+7, 5+7, and 6+7, with a mode at 5+7. The total ray count ranging from 20 to 27 occurred in nine combinations from 4+5:3+7 to 6+7:6+7, with the same mode. Right-side counts (9–13) showed six combinations from 4+5 to 6+7, while left-side counts (10–13) were in five combinations from 3+7 to 6+7.

Esox reicherti

Too few specimens were available to make any comparisons of the detailed counts of this species with the others. The four individuals examined all had different combinations as follows: 5+8:5+8, 6+7:5+8, 6+7:6+8, 6+8:5+8. It appears that symmetry in this species would be in combinations of 5+8 and 6+7 with 5+8 probably a component of any modal count since it occurs most often here. In many ways this species fits into the pickerel group in branchiostegal characteristics and would likely have a small number of combinations of counts.

Sidedness

Concurrent with bilateral asymmetry and variations of this type in fishes is a tendency toward right- or left-sidedness. This is most obvious in Heterosomata. Throughout the Holostei and Teleostei, in fishes in which the branchiostegal membranes are not united, the left membrane generally overlaps the right to a slight degree over the whole length. However, in those groups with high numbers of meristic parts there is a greater degree of variation in this respect. In the family Esocidae most individuals are left-sided individuals (left membrane overlaps right) but the occurrence of right-sidedness is variable and occurs with greater frequency in the pickerels than in the two larger species, the pike and the muskellunge.

Table II gives sidedness of individuals of each species examined as the number of dextral and sinistral individuals as indicated by overlap of branchiostegal membranes.

In large numbers examined of the two larger species no right-handed individuals were seen. In the pickerels the numbers examined were too small to subject to statistical tests, but 1 (or 3%) of *E. niger* were right-handed as were 1 (or 1.5%) of the *E. americanus* and none of the *E. vermiculatus* or *E.*

TABLE II

Amount and direction of asymmetry and sidedness in Esocidae
(after Hubbs and Hubbs (1945 (1))

Species	Sidedness by overlap		Amount of asymmetry			Direction of asymmetry as degree sinistrality		
	No. sinistral	No. dextral	Ant. rays	Post. rays	Total rays	Ant. rays	Post. rays	Total rays
Esox masquinongy	70	0	51	35	53	78	28	73
E. lucius	150	0	32	11	34	55	56	59
E. niger	31	1 (3%)	38	19	28	50	67	67
E. americanus	63	1 (1.5%)	53	34	37	59	23	42
E. vermiculatus	20	0	40	20	35	75	50	86
*E. vermiculatus**	442	32 (7%)	40	21	34	68	33	63

*An interpopulation count from Hubbs and Hubbs (1945 (1)).

reicherti. However, in the interpopulation sample of 442 *E. vermiculatus* that Hubbs and Hubbs (1945 (1)) examined 32 (or 7%) were right-handed.

Besides the visible characteristic of overlap of the membranes there is sidedness involved in the greater number of rays on one side.

In their study of asymmetry and variability Hubbs and Hubbs (1945 (1)) found that 80% of *Oncorhynchus tshawytscha* examined had one or two more rays on the left side. They claimed that in *E. vermiculatus* the differences (sidedness) are apparent but require long statistical analysis for reliable demonstration. They do mention, however, that in *E. vermiculatus* when the left membrane overlaps the right the total number of rays on the left is greater than that on the right. When the hyoid segment counts are considered, posterior rays are more numerous on the right than on the left but anterior rays are strongly enough sinistral to make the total count "slightly but significantly higher on the left side". Hubbs and Hubbs used formulae to express the asymmetry as amount and direction as follows:

$$\text{Amount of asymmetry} = \{100\ (L + R)\}/N$$
$$\text{Direction of asymmetry (as degree sinistral)} = 100\ L/(L + R)$$

where L and R are the number of individuals having greater development on that side (number of rays) and N is the total number in the sample.

Values according to these formulae are given for the various species of the family Esocidae (see Table II).

It can be seen from these values that all species have a moderately high degree of asymmetry (amount for total rays) and that the anterior rays consistently exhibit the greater amount of asymmetry. The direction of asymmetry expressed as degree of sinistrality also shows that the anterior rays are the ones which create the strong left-handed effect reflected in the total ray values for direction.

While the pickerel tended to show higher numbers of right-handed individuals than did the larger species, there was no obvious cline in this tendency indicated in the direction values for all the species. In fact, *E. vermiculatus*, in which

Hubbs and Hubbs found 7% of the sample were right-handed, shows a degree of left-handedness as high or higher than the two large species (*E. masquinongy* and *E. lucius*) in which no right-handed individuals were seen. While the calculations for direction do not coincide, there is strong agreement between values for quantity obtained by Hubbs and Hubbs on an interpopulation level and the results of the intrapopulation samples for the present study.

Variation and Bilateral Asymmetry in Arrangement of Branchiostegal Rays

As has been described above, the anterior and posterior rays have a definite arrangement on the hyoid segments. The anterior rays arise from the dorsal surface of the epihyal and the posterior rays from the ventral surface of the ceratohyal.

Commonly the rays are equally spaced except at the separation between the segments where a wider gap helps distinguish the rays of the two segments. Normally all rays are firmly but flexibly fixed by connective tissue to the surface of the hyoid segments. The arrangement of rays most often found is shown in Fig. 1. In the specimens examined, however, there appeared, repeatedly, six types of aberrations of arrangement which seemed by their constant reappearance to be the result of differential development rather than injury. Aberrations obviously due to injury also appeared. The former aberrations, when they appeared, were constant enough to classify them by number and tabulate the frequency, in each species, that each type occurred on the right, left, or both sides (see Table IV).

The aberrations which appeared often enough to classify are described in Table III and shown in Fig. 2.

The most frequent types of aberrations or instances of bilateral asymmetry in arrangement of rays were types 1 and 2 in which two or more rays approxi-

TABLE III

Classification and description of aberrations in arrangement of branchiostegal rays in family Esocidae

Assigned type No.	Description of aberration
1	First and second anterior rays closely adhering for at least one-half their length from base so as to appear like a single, branched ray. First ray often not firmly attached to bone
2	Anterior rays other than the first and second closely adhering so as to appear like a single, branched ray
3	First anterior ray normally spaced but short and not connected to hyoid bone
4	First anterior ray normally spaced but touching the second ray one-quarter to one-half the distance from the base of the second; or the first recurved at this point behind the second
5	First posterior ray arising on ventral surface of hyoid bone but on anterior segment or at least on hinge ligament
6	First anterior ray entremely thick, equal in thickness to two normal first anterior rays

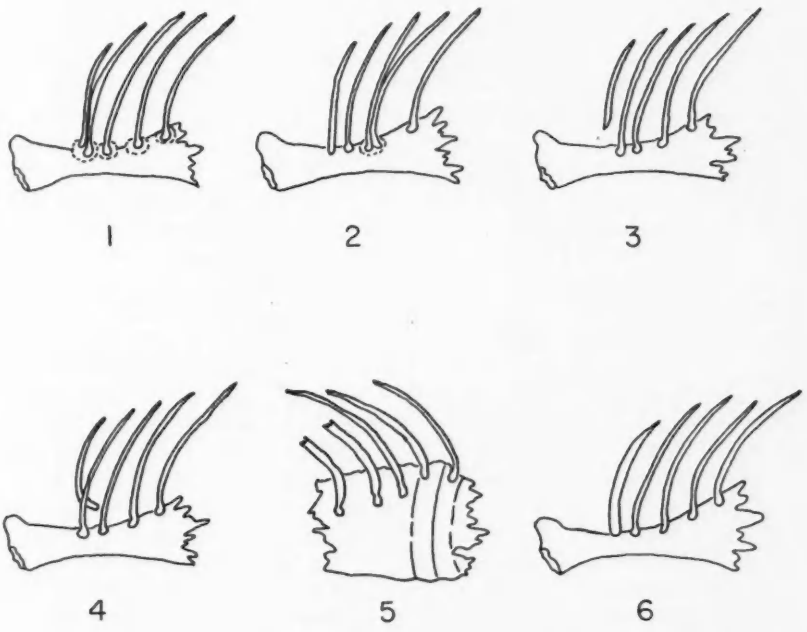

FIG. 2. Sections of hyoid bones of *Esox lucius* showing six types of common aberrations in arrangement of branchiostegal rays in family Esocidae. See Table III for description.

mated and appeared superficially as a single, branched ray (see Table IV). They could, however, be teased apart and traced as separate rays to their bases. These two types occurred a total of 92 times (27%) in the 336 specimens included in this study. These two types of aberrations occurred most frequently in the two large species *E. masquinongy* and *E. lucius*. Even in ray arrangement, left-sidedness occurred. Deviations in arrangement occurred more often on the left side than on the right side and in only 18 of 117 occurrences of the various types were the aberrations bilaterally symmetrical. In 99 cases the deviation occurred only on one side and in 48% of these cases it was on the left side only and only 32% of the deviations were on the right side only. Except for a single occurrence of type 5 in *E. masquinongy* there were more aberrations of each type in each species on the left alone than on the right or both sides.

As was mentioned above, the greatest frequency of the greatest number of types of these aberrations appeared in the larger species. *E. lucius* showed the greatest frequency but *E. masquinongy* exhibited more types. *E. niger* showed only five of type 1. *E. vermiculatus* showed no aberrations of arrangement so that the population of *E. americanus* which has 10 aberrations of three types stood out as the only pickerel with large numbers of aberrations.

TABLE IV

Frequency of various types of aberrations of arrangement of branchiostegal rays in the family Esocidae

		Type																	
		1			2			3			4			5			6		
		Side																	
	N	R	L	RL	R	L	RL	R	L	RL	R	L	RL	R	L	RL	R	L	RL
E. masquinongy	70	9	11	9	1			1	5	3	1	4		1				2	
E. lucius	150	20	22	5	1	3		1	1		1							1	
E. niger	32		5																
E. americanus	64		1		1	5	1		1		1								
E. vermiculatus	20	No aberrations of arrangement																	
Totals	336	29	39	14	3	8	1	2	7	3	3	4	0	1	0	0	0	3	0

Conclusions

Branchiostegal rays have long been used to separate various species of the family Esocidae. When total counts (both sides) or single-side counts are used the value of this character breaks down. However, if the number of rays arising from each ramus of the hyoid on each side are counted this count may further separate the species and remove overlap.

As in vertebrates in general, most fishes appear superficially to be symmetrical about the sagittal axis. In those fishes with large numbers of meristic parts this is often not true. Various species of the family Esocidae exhibit different and varying amounts of bilateral asymmetry in the branchiostegal membranes. Differential development leads to one membrane overlapping the other. Usually left overlaps right; however, this is sometimes reversed. The number of rays associated with each ramus of the hyoid and the arrangement of these rays exhibit bilateral asymmetry.

Since the number of rays can vary from one year class to another in the same population (see Appendix Table III), the rate of embryological development must control to a certain degree the number of rays found. The final number may be strongly affected by the space available when their development is initiated. The slower the development the greater the eventual number. The role of rate of development in determining the ray number may be more apparent if eggs of each species were hatched and the young all reared under constant conditions of temperature and possibly light. In some way this mechanism must also control the development, on each side, of the muscles overlaying the branchiostegals and this in turn possibly determines the membrane which will overlap. Hubbs and Hubbs (1945 (1)) stated that the direction of overlap of the membranes and the different muscular development along the hyoid arches of the two sides seem to be more significant than leftness or rightness as such, as a correlative of ray number. Why there is a far greater tendency for the left rather than the right side of these species to develop more rays and also overlap is at present unknown.

References

1. Hubbs, C. L. and Hubbs, L. C. Bilateral asymmetry and bilateral variation in fishes. Papers Mich. Acad. Sci. **30**, 229–310 (1945).
2. Scott, W. B. A checklist of the freshwater fishes of Canada and Alaska. Roy. Ontario Museum, Div. Zool. Palaeontol. 1–30 (1958.)

Appendix follows.

Appendix

APPENDIX TABLE I

Frequency distribution of symmetrical branchiostegal ray count combinations for intrapopulation samples of species of family Esocidae

E. masquinongy (70)						
R.A+P	7+9	8+9	8+10	9+9	9+10	
L.A+P	7+9	8+9	8+10	9+9	9+10	T
F	1	4	8	3	1	17

E. lucius (150)								
R.A+P	6+7	6+8	6+9	7+7	7+8	8+7	8+8	
L.A+P	6+7	6+8	6+9	7+7	7+8	8+7	8+8	T
F	1	10	1	1	50	1	3	67

E. niger (32)				
R.A+P	6+8	6+9	7+9	
L.A+P	6+8	6+9	7+9	T
F	1	13	3	17

E. americanus (64)							
R.A+P	4+7	4+8	5+7	5+8	6+7	6+8	
L.A+P	4+7	4+8	5+7	5+8	6+7	6+8	T
F	1	2	2	6	2	9	22

E. vermiculatus (20)				
R.A+P	4+7	5+7	6+7	
L.A+P	4+7	5+7	6+7	T
F	2	8	1	11

APPENDIX TABLE II

Frequency distribution of all two side combinations of anterior and posterior ray counts for intrapopulation samples of the species of family Esocidae

E. masquinongy (70)

R.A+P	7+9	7+9	7+9	7+9	7+10	7+10	7+10	7+10	7+10	8+9	8+9	8+9
L.A+P	7+9	8+8	8+9	9+9	6+10	8+9	8+10	9+9	9+10	7+9	8+9	8+10
F	1	1	1	1	1	3	4	3	1	2	6	5
R.A+P	8+9	8+10	8+10	8+10	8+10	8+10	8+10	9+9	9+9	9+9	9+10	
L.A+P	9+9	7+10	8+9	8+10	9+9	9+10	10+10	8+10	8+11	9+9	9+10	
F	9	1	6	12	3	1	1	1	1	3	1	

E. lucius (150)

R.A+P	5+8	6+7	6+7	6+8	6+8	6+8	6+8	6+8	6+9	6+9	7+7	7+7	7+7	7+7	7+7
L.A+P	7+7	6+7	6+8	5+8	6+8	6+9	7+8	8+8	6+9	7+8	5+8	6+8	7+7	7+8	8+7
F	1	1	1	1	11	1	9	1	1	1	2	1	1	1	1
R.A+P	7+8	7+8	7+8	7+8	7+8	7+8	7+9	8+6	8+6	8+7	8+7	8+8	8+8	8+8	9+7
L.A+P	5+8	6+8	7+7	7+8	8+7	8+8	7+8	7+8	8+8	7+8	8+7	6+8	7+8	8+8	7+9
F	2	9	2	73	1	11	2	1	1	1	1	2	4	5	1

E. niger (32)

R.A+P	5+9	5+9	6+8	6+8	6+9	6+9	6+10	
L.A+P	5+9	6+9	6+8	7+8	6+9	7+9	6+9	
F	1	1	1	1	14	2	1	
R.A+P	6+10	6+10	7+7	7+8	7+9	7+9	8+9	
L.A+P	7+9	7+10	6+9	6+9	6+9	7+9	7+10	
F	1	1	1	2	2	3	1	

E. americanus (64)

R.A+P	4+7	4+8	4+8	4+8	4+8	4+9	5+7	5+7	5+7	5+8	5+8	5+8	5+8	5+8
L.A+P	4+7	4+8	5+7	5+8	6+7	5+9	4+8	5+7	6+7	4+8	4+9	5+7	5+8	5+9
F	1	2	4	1	1	1	2	3	3	1	1	2	7	1
R.A+P	5+8	5+8	5+9	5+9	6+7	6+7	6+7	6+8	6+8	6+8	7+8	7+8	7+8	7+8
L.A+P	5+7	5+8	5+8	6+8	5+7	5+8	6+7	5+8	6+8	7+7	4+9	6+8	7+7	7+8
F	4	3	1	2	2	1	3	4	9	1	1	1	1	1

E. vermiculatus (20)

R.A+P	4+5	4+7	4+7	5+6	5+7	5+7	5+7	5+8	6+7	
L.A+P	3+7	4+7	5+7	4+7	5+6	5+7	6+7	6+7	6+7	
F	1	2	4	1	1	8	1	1	1	

APPENDIX TABLE III

Frequency of distribution of total branchiostegal ray count and single-side counts in an intrapopulation sample of *E. masquinongy* and in two year classes comprising this sample

Total count	32	33	34	35	36	37	38	N	$\bar{x}$
Total sample	2	4	10	28	22	2	2	70	35.1
Year class A		1	4	9	18	2	2	36	35.6
Year class B	2	3	6	19	4	—	—	34	34.5
Right side	**16**	**17**	**18**	**19**	**20**				
Total sample	4	34	31	1	—			70	17.4
Year class A	1	13	21	1	—			36	17.1
Year class B	3	21	10	—	—			34	17.2
Left side									
Total sample	5	17	43	4	1			70	17.7
Year class A	1	4	26	4	1			36	18.0
Year class B	4	13	17	—	—			34	16.8

ON SOME ORNITHOPHILIC BLOOD-SUCKING DIPTERA IN ALGONQUIN PARK, ONTARIO, CANADA[1]

GORDON F. BENNETT

Abstract

A useful technique was devised for the collection of biting Diptera after they had fed on various birds exposed in dissimilar habitats in Algonquin Park. As a result, the following species of ornithophilic Simuliidae and Ceratopogonidae, including four new species and other species the feeding habits of which were previously unknown, were collected: *Simulium aureum.*, *"latipes"*, *quebecense*, *croxtoni*, *rugglesi*, *subexcisum* (Edw. of Twinn, 1936), *Simulium* "H", *Simulium* (*Eusimulium*) n.sp., *Prosimulium decemarticulatum*, *Cnephia invenusta*, *Cnephia* "U", *Culicoides sphagnumensis*, *stilobezzioides*, *Culicoides* n.sp. near *piliferus*. Several species of Culicidae and Tabanidae which would also feed on birds are listed.

The data further indicated that the simuliids and ceratopogonids showed host and habitat preferences; these preferences were particularly marked among certain species such as *Simulium rugglesi* and *Culicoides* n.sp. near *piliferus*. The simuliids occurred commonly from May 20 through mid-July; ceratopogonids from June 2 through mid-July. Woodland simuliids usually fed at dusk while lake shore simuliids fed in the early evening; biting midges were crepuscular to nocturnal. Other miscellaneous observations on feeding behavior are included.

Introduction

The feeding behavior of several species of mosquitoes has been studied in relation to the animals on which they feed, particularly those which feed on man. Much less is known concerning the feeding behavior of most species of simuliids and biting midges, especially those that feed on, and in some instances transmit parasites to, animals other than man. This became apparent during recent investigations on the life history of various avian blood parasites. For example, most species of simuliids taken from birds were not recovered on mammals. Moreover, certain of these ornithophilic simuliids appeared to prefer some birds more than others. Similar differences were noted among a few species of *Culicoides* (Diptera:Ceratopogonidae). Collections were made, therefore, of the blood-sucking Diptera that fed on various birds exposed in different habitats at different times. The results of these observations are reported herein. The simuliids and ceratopogonids are considered in most detail although a few observations on mosquitoes and tabanids are included.

Materials and Methods

Collections of ornithophilic biting flies were made in May, June, and July during the years 1956–59 at the Wildlife Research Station of the Ontario Department of Lands and Forests in Algonquin Park. Collections were made from various birds exposed in the four following habitats on or near Lake Sasajewan: (i) on the grassy shore within 15 feet of the lake (Fig. 1) or,

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A contribution from the Department of Parasitology, Ontario Research Foundation, Toronto, Ontario.

occasionally, on a raft 25–40 feet off shore; (ii) on the same lake shore but 15 feet in the air; (iii) on the forest floor of an open mixed conifer–birch forest approximately 200 feet from the North Madawaska river and about half a mile from the previous sites; (iv) 5 to 25 feet in the air (Fig. 2) near or directly over the sites used in (iii).

The following birds (9), with the exception of the woodpeckers, were maintained in captivity: Mallard duck, *Anas platyrynchos;* black duck, *Anas rubripes;* domestic duck (white Pekin), *Anas boschas;* ruffed grouse, *Bonasa umbellus;* domestic fowl, *Gallus domesticus;* raven, *Corvus corax;* crow, *Corvus brachyrynchos;* blue jay, *Cyanocitta cristata;* Canada jay, *Perisoreus canadensis;* bronzed grackle, *Quiscalus versicolor;* robin, *Turdus migratorius;* great blue heron, *Ardea herodias;* hairy woodpecker, *Dendrocopus villosus;* yellow-bellied sapsucker, *Sphyrapicus varius;* yellow-shafted flicker, *Colaptes auratus;* Arctic three-toed woodpecker, *Picoides arcticus;* saw-whet owl, *Aegalius acadica;* sharp-shinned hawk, *Accipiter striatus;* white-throated sparrow, *Zonotrichia albicollis;* purple finch, *Carpodacus purpureus.*

The birds, depending on their size, were confined in cages either 8- or 12-inches cube. The sides and tops of these cages were covered with chicken wire or heavy fish net ($\frac{1}{2}$-in. or 1-in. mesh) and the bottoms with $\frac{1}{2}$-in.-mesh hardware cloth. Hoods, made of a dark cloth or chamois, were put over the heads of exposed birds (except ducks and sparrows). The hooded birds remained relatively motionless in these exposure cages; flies were able to feed on them without being dislodged. Small birds, such as the sparrows and finches, were placed in small tubes of $\frac{1}{2}$-in.-mesh chicken wire. Each tube was then compressed firmly about the bird to prevent its movement. This seemed more satisfactory for small birds than hoods. The same bird was seldom used on 2 successive days to avoid placing too much stress on any one of them. Domestic and tame black ducklings, especially if used in pairs, soon became relatively quiet without the use of hoods. Adult domestic ducks, used singly, also adjusted quickly to exposure cages.

The exposure cages containing the birds were placed on 2-ft squares of white plywood at the test sites (Fig. 1). The birds were then exposed for 20–30 minutes, following which they were covered with a collecting cage. The collecting cage consisted of a frame (2 ft × 2 ft × 2 ft) covered with nylon screen (60 meshes to the inch) on five sides, leaving the bottom open. Strips of plastic or rubber sponge glued to the bottom frame of the cage formed a tight seal and prevented escape of the flies. The birds were left under these collecting cages for 20–30 minutes. Only engorged flies which had left the bird and settled on the sides of the cages were collected. Flies were collected in aspirators through a sleeve at the top of the cage.

The procedure was modified slightly for collecting flies from different heights. The birds, in the exposure cages, were raised to various heights by means of a simple rope and pulley system (Fig. 2). A four-point attachment of two lift ropes to the exposure cage prevented it from spinning in the air. The cages were raised to the desired level in 10–15 seconds and left there

PLATE I

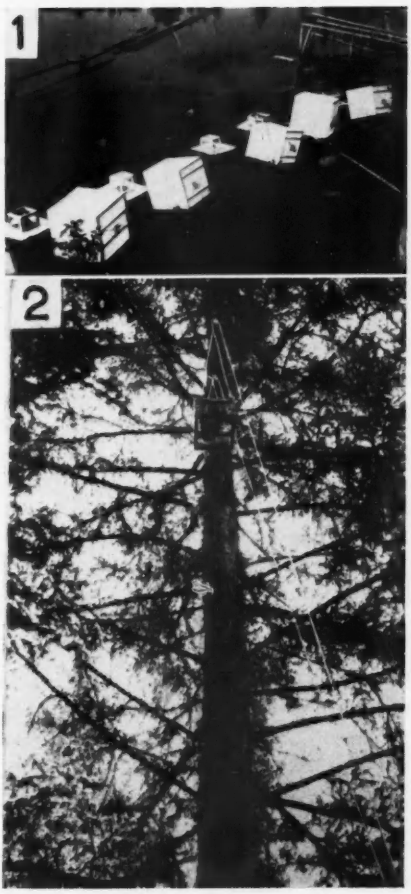

FIG. 1. Birds exposed in cages placed on the white plywood squares along the lake shore; the collecting cages are nearby.
FIG. 2. Bird exposed in cage 20 feet from the ground in woodland habitat.

for 20–30 minutes. They were then lowered carefully (in 30–40 seconds) to the plywood squares and the engorged flies were collected as previously described.

The engorged flies, following removal from the cages, were either stored in alcohol or pinned and dried by a quick-freeze method developed by Wood (12). Many of the Simuliidae were identified by Mr. Guy Shewell and the *Culicoides* by Mr. J. A. Downes. They kindly assisted the author with the identification of the remainder.

The number of collections given in Tables I, IV, V, and VII are presented to illustrate relative collecting effort. It does not follow that each species of fly was taken in every collection as collections were made when some species of flies were absent.

Results

Species of Simuliidae and Ceratopogonidae

Examination of the biting flies collected from various birds showed that species of Simuliidae and Ceratopogonidae were most common; a few species of Culicidae and Tabanidae were also collected.

Among the Simuliidae taken from the birds were representatives of the following clearly defined taxa: *Prosimulium* (*Helodon*) *decemarticulatum* Tw., *Simulium* (*Byssodon*) *rugglesi* N. & M., *Simulium* (*Simulium*) *venustum* Say, and *Simulium* (*Simulium*) *decorum* Wlk.

Revision in the identification of some of the remaining species is expected following the taxonomic studies of the genus that are now in progress by Shewell, Wood, and others. Dunbar (5) recognizes seven cytogenetically distinct forms of *S.* (*Eusimulium*) *aureum* Fries. Consequently, specimens identified herein as *S. aureum* may include more than one species. Possibly this has little significance in the present investigation for no distinct patterns of feeding behavior, which might suggest species groups, were noted among the specimens of *aureum* collected.

Specimens designated *Simulium* (*Eusimulium*) *latipes* Meigen in this study also include an undetermined number of *Simulium* (*Eusimulium*) *quebecense* Twinn; herein the term "*latipes*" refers to both species. The complexity of this question is further increased by the fact that Peterson (8) believes that *latipes* Meigen is restricted to Europe.

A new species of *Simulium* (*Eusimulium*), herein termed *S.* (*Eusimulium*) "H" pending Wood's description and naming of it, was taken in numbers during 1956–57. *S.* (*Eusimulium*) "H" is similar in appearance to *Simulium* (*Eusimulium*) *croxtoni* N. & M. and *S. euryadminiculum* Davies (= *Simulium* (*Hellichia*) *canonicolum* D. & S.). Confusion between these three species has probably led to erroneous conclusions in the past. For example, *S. croxtoni* and *S. euryadminiculum* were said to be involved in the transmission of *Leucocytozoon simondi* and *Ornithofilaria fallisensis* (1,6). Specimens collected at the same time and from the same host, bearing the determination labels of *S. croxtoni* and *S. euryadminiculum*, have been re-examined and identified

as *Simulium* "H". Another new species of *Simulium* (*Eusimulium*) obtained in low numbers is herein termed *Simulium* (*Eusimulium*) (*subexcisum* Edw. of Twinn, 1936). Wood (12) has also detected a third undescribed species of *Simulium* (*Eusimulium*) in the 1959 collections. Two species of *Cnephia*, namely *Cnephia* (*Ectemnia*) *invenusta* Wlk. and *Cnephia* (*Cnephia*) "U" (which is being described by Guy E. Shewell), were collected.

It is interesting to note that all the ornithophilic Simuliidae encountered in this study possessed the bifid tarsal claw. This observation supports Shewell's (10) prediction that this type of tarsal claw is an adaptation for feeding on birds.

Mr. Downes has identified the following six species of *Culicoides* that had fed on birds: *C. sphagnumensis* Williams, *C. stilobezzioides* Foote and Pratt, *C. haematopotus* Malloch, *C. obsoletus* (Meigen), *C. crepuscularis* Malloch, *Culicoides* n. sp. near *piliferus* Root and Hoffman.

Distribution by Habitat

Preliminary observations suggested that some species of flies were more abundant in some habitats than others. Evidence to support or disprove this view was obtained by collecting biting flies from birds exposed in different habitats. The results (Table I) indicated that certain flies, viz., *rugglesi*, *subexcisum* (Edw. of Twinn, 1936), *Simulium* "H", *Simulium* n. sp., *Culicoides* n. sp., were usually collected at the ground level at the lake shore; the rest were generally confined to the forest habitats.

As *rugglesi* was taken most frequently at ground level near water (Table I), experiments were carried out to determine the distribution of *rugglesi* by habitat more precisely. *S. rugglesi* showed almost ideal characteristics for such

TABLE I

Number of engorged flies captured from various birds in different habitats, 1957–59

	Lake shore, ground			Lake shore, 15 ft in air			Forest, ground			Forest, 5–25 ft in air		
	1957 (200)*	1958 (119)	1959 (88)	1957 (0)	1958 (18)	1959 (0)	1957 (80)	1958 (46)	1959 (0)	1957 (0)	1958 (150)	1959 (185)
Simuliidae												
aureum	1	7	5		27		217	20			732	356
decemarticulatum	0	1	0		0		0	5			546	504
"latipes"	1	7	20		1		122	79			1096	877
croxtoni	0	2	0		1		14	1			99	151
subexcisum (Edw. of Twinn 1936)	0	5	6		0		0	0			0	0
Simulium "H"	155	8	225		9		1	0			0	0
Simulium n.sp.	0	0	10		0		0	0			0	0
rugglesi	739	784	2150		15		75	10			0	0
venustum	3	39	9		0		2	5			1	0
decorum	0	0	0		0		1	0			1	0
invenusta	0	1	0		0		0	0			2	3
Cnephia "U"	0	0	0		0		0	0			13	176
canonicolum	0	0	9		0		0	0			0	0
Ceratopogonidae												
sphagnumensis	1	0			0		6	26			1160	
stilobezzioides	1	0			0		6	2			124	
crepuscularis	5	0			0		3	5			61	
obsoletus	1	22			0		109	0			11	
haematopotus	2	0			0		7	1			8	
Culicoides n.sp. near *piliferus*	121	1000's			0		10	6			1	

No collections

No tabulation

*Number of collections in parentheses.

study. (i) It occurred in relatively large numbers. (ii) It was present for most of the black fly season. (iii) The ducks, of same size and species, from which *rugglesi* were collected were abundant and easy to maintain. Eight pairs of ducks were placed at approximately 50-foot intervals in a line extending from the lake shore into the forest. The experiment was repeated on 2 days. In the first trial, 95% of 478 *rugglesi* were taken in the first 50 feet and 1.6% of the flies between 300 and 400 feet from the water. In the second trial, 71.4% of 330 flies were taken in the first 50 feet, while only 2.4% of the total were taken 300 to 400 feet in the forest. These results support those in Table I, indicating *rugglesi* is obtained in a narrow zone along the water's edge.

Most *aureum*, "*latipes*", *croxtoni*, and *decemarticulatum* were taken above ground in the forest, and are referred to as "woodland" species (Table I). Collections were made from crows exposed at different heights to determine whether the woodland species show preferences for specific elevations in the forest. Simultaneous collections were made from crows at four levels, a number of collections at each level being made each evening. The results (Table II) support the previous view that most woodland species feed on birds several feet above ground level; no evidence of a preferred stratum between 5 feet and 20 feet was obtained.

In general, the data indicate that most of the ornithophilic flies taken in these collections were in one of two habitats. The first habitat type, consisting of the lake shore and open water, was preferred by *rugglesi*, *subexcisum* (Edw. of Twinn, 1936), *Simulium* "H", and *Culicoides* near *piliferus* (Table I). A few specimens of these flies were taken at ground level in the forest and even fewer at 15 feet in the air along the lake shore.

The second habitat consisted of the middle levels of the forest. The majority of the following flies were taken in this habitat: *aureum*, "*latipes*", *croxtoni*, *invenusta*, *Cnephia* "U", *decemarticulatum*, *sphagnumensis*, *stilobezzioides*, *crepuscularis*, and *haematopotus* (Table I). Some overlapping into other habitats occurs, particularly on to the forest floor and at 15 feet elevation

TABLE II

Engorged Diptera collected in simultaneous exposures of crows at four heights above the ground in the forest habitat

Species	Heights from ground, ft			
	0	5	10	20
Simuliidae				
aureum	1	9	8	36
"*latipes*"	23	54	53	17
croxtoni	1	8	5	19
decemarticulatum	2	12	45	9
Culicoides				
sphagnumensis	24	280	360	160
stilobezzioides	1	16	21	18
crepuscularis	5	5	6	17
haematopotus	1	1	0	3
obsoletus	0	0	4	5

along the lake shore. Although the number of collections in each of these habitats differs, consistently more flies were taken in the middle levels of the forest habitat. For example, *aureum*, "*latipes*", and *decemarticulatum* occur in the lake shore (15 feet from the ground), the forest floor, and the forest canopy habitats. Although twice as many collections were made in the latter habitat, more than 10 times as many flies were taken than in the former two habitats. Restricted distribution shown by such species as *rugglesi* and *decemarticulatum* suggests that intensive collecting in habitats other than those tested may reveal species which are scarce or absent in the present collections.

Host Preference

Incidental observations prior to this study suggested that some species of flies fed on certain birds more often than others. Collections of flies were made, therefore, from different birds exposed in the same habitat at the same time to determine the validity of such a conclusion.

It had been shown (1, 6) that *rugglesi* feeds on ducks but the extent to which it feeds on other birds was unknown. The following birds, therefore, were exposed along the lake shore: one domestic duck, four crows, two grouse, two robins, three white-throated sparrows, and a saw-whet owl. Four collections of flies were made from each bird with the following results: 100 *rugglesi* from the single domestic duck, 5 from the grouse, and 1 from the four crows; no flies were taken from the other birds. In another experiment, using four crows, two grouse, and one domestic duck, four collections from each of these species were made. Ninety-five *rugglesi* were taken from the duck, five from the grouse, and one from the crows. Obviously, *rugglesi* feeds on ducks in preference to the other species of birds exposed at the same time. In another experiment, using a duck, a crow, and a grackle, 81 *Eusimulium* "H" were taken from the duck, but only 3 from the grackle and none from the crow.

To determine whether *S. rugglesi* showed a preference for one species of duck more than another, the number of flies from wild and domestic ducks, of approximately the same size, exposed simultaneously, were compared. In 18 collections from domestic ducks, 479 *rugglesi* were obtained, an average of 26 flies per duck per collection with a range of 7–40 flies in a collection. Only 68 *rugglesi* (6.8 flies/duck/collection) were taken in 10 collections from the black duck and 106 flies (10.6 flies/duck/collection) in 10 collections from the mallard. More *rugglesi* were taken from the domestic than the wild ducks, suggesting that even among birds of the same genus (*Anas*), the flies will select one species in preference to another.

Similar collections of flies were made from birds exposed in the woodland habitats to determine whether there were indications of host specificity. Domestic ducks, grouse, a blue jay, robins, and a sharp-shinned hawk were exposed in the same habitat on 2 evenings to the biting activity of the flies. Three collections were made each evening from these birds (Table III) which were exposed at 15–20 feet above the ground. During another evening, the biting flies were collected from three ducks and two grouse exposed under the

TABLE III

Engorged Simuliidae obtained from different birds exposed at the same time in woodland habitat

Experiment	Bird		*aur.*	*"lat."*	*decem.*	*crox.*	Miscellaneous Simuliidae	Total flies
	Species	Total						
1	Ducks	(3)	3	28	9	0	0	40
	Grouse	(1)	162	178	38	0	0	378
	Blue jay	(1)	15	21	5	0	4	45
	Robin	(2)	18	50	5	0	2	75
	Sharp-shinned hawk	(1)	13	80	2	0	1	96
2	Ducks	(3)	3	34	9	0	1	47
	Grouse	(2)	43	131	38	0	11	223
3	Ducks	(2)	0	9	9	8	0	26
	Grouse	(1)	11	68	22	9	0	110
	White-throated sparrow	(1)	2	13	5	1	1	22
	Crow	(1)	38	52	0	8	0	98
	Grackle	(1)	6	18	2	2	1	29
	Robin	(1)	2	12	3	5	2	24
4	Domestic duck	(1)	0	2	0	0	0	2
	Black duck	(1)	0	2	9	1	0	12
	Mallard duck	(1)	0	17	12	2	0	31
	Grouse	(1)	0	21	35	9	0	65
5	Duck	(1)	2	0	0	0	0	2
	Crow	(1)	4	0	8	4	4	20
	Grouse	(1)	16	6	7	0	3	33

same conditions. These and other experiments (Table III) indicated that the woodland Simuliidae preferred the woodland birds that were tested rather than the ducks. Significantly in all these experiments in the woodland habitats, hundreds of unfed woodland simuliids were present about the ducks. On the other hand, few unfed flies were taken in the collections from the woodland birds.

The species of flies taken from the various birds in all the collections (Tables IV, V) appear to fall into two groups. *S. rugglesi, subexcisum, Simulium* "H", and *Culicoides* near *piliferus* were taken most frequently on ducks; several specimens of *Culicoides* near *piliferus* were taken also on the great blue heron (Table IV). Relatively few of these species of flies were taken on other species of birds, although they were frequently exposed in the lake shore habitat.

S. aureum, "latipes", croxtoni, P. decemarticulatum, Cnephia invenusta, Cnephia "U", *Culicoides sphagnumensis, stilobezzioides,* and *crepuscularis* occur on the woodland birds (Tables IV, V). Some individual differences among the woodland birds are noted, although more information is required to be able to state host preference with certainty. *S. aureum* occurred frequently on most birds except crows; *croxtoni* occurred commonly on grouse and crows while *"latipes"* and *Cnephia* "U" were ubiquitous. Among the *Culicoides, sphagnumensis* was collected frequently from the larger birds; *stilobezzioides* was ubiquitous.

Several species, such as *Cnephia invenusta* and *Simulium* (*subexcisum* Edw. of Twinn, 1936), were taken in small numbers. These low numbers may

TABLE IV

Diptera captured after feeding on various birds, 1957

	Dom. duck	Grackle	Ruffed grouse	White-throated sparrow	Blue jay	Raven	Purple finch	Great blue heron	Canada jay
	No. collections								
	285	47	80	41	32	41	13	14	20
Simuliidae									
Eusimulium "H"	150	0	0	4	0	0	0	0	1
aureum	32	8	69	3	3	89	0	16	0
"latipes"	24	15	24	7	17	38	1	4	2
croxtoni	6	1	1	6	0	0	0	0	0
rugglesi	1000	1	3	0	0	0	0	2	0
venustum	4	0	0	0	0	0	0	0	0
decorum	1	0	0	0	0	0	0	0	0
Ceratopogonidae									
sphagnumensis	3	1	2	1	0	1	0	0	0
stilobezzioides	1	1	0	1	0	4	0	1	0
crepuscularis	1	1	0	1	0	0	2	2	0
obsoletus	44	21	3	4	2	39	0	0	0
haematopotus	5	2	1	2	1	0	0	4	0
n.sp. near *piliferus*	Hundreds per collection	4	23	5	3	0	12	51	3
Culicidae									
Mansonia perturbans	32	9	2	0	0	60	0	23	0
Anopheles walkeri	2	0	0	0	0	1	0	0	0
Aedes intrudens	1	0	2	0	1	2	0	0	0
Aedes canadensis	3	0	1	1	0	6	0	0	0
Aedes abserratus	1	0	0	0	1	0	0	0	0
Aedes aurifer	0	0	1	0	0	0	0	0	0

be indicative of a small population in the area, but it may also indicate that the collections were made in habitats and/or with hosts not suitable for these species. *S. venustum*, *decorum*, and *Culicoides obsoletus*, which are known to feed on mammals, were occasionally taken on birds, generally the larger ones (Tables IV, V). Few were taken from birds, however, compared to the hundreds that were feeding on man at the same time.

Few *Culicoides* were captured feeding on ruffed grouse (Tables IV, V). In 1959, several hundred biting midges, mainly *sphagnumensis* and *stilobezzioides*, were taken each night for 5 nights from a spruce grouse (*Canachites canadensis*). Although a different species of grouse, these collections show that biting midges will feed commonly on members of the Tetraonidae. Few Ceratopogonidae were captured from robins (Tables IV, V) although in 1959, during each of 3 nights in early July, at least 200 biting midges were collected from two robins.

Differences were noted in the number of flies feeding on different species of woodland birds. In 12 simultaneous collections from blue and gray jays, a total of 300 Simuliidae were collected from blue jays, only 41 from gray jays. Similarly, 47 simuliids were taken from robins, none from an Arctic three-toed woodpecker; 114 black flies from robin, 56 from a hairy woodpecker; and 77 simuliids from robin, 32 from a yellow-bellied sapsucker. Clearly, more flies fed on the robins than on the woodpeckers.

It is important that the feeding behavior of ornithophilic biting flies be more clearly understood as species of these flies are now known to be the vectors of

TABLE V

Diptera captured after engorgement on various birds, 1958–59

	Ruffed grouse	Crow	Raven	Domestic duck	Robin	Blue jay	Saw-whet owl	Sharp-shinned hawk	White-throated sparrow	Flicker	Grackle	Purple finch	Canada jay
	No. collections												
	66 (27)*	60 (27)	13	122 (83)	27 (44)	18 (28)	13	5	15 (4)	7	(13)	(12)	(12)
Simuliidae													
aureum	543 (125)*	74 (19)	24	12 (10)	18 (76)	22 (73)	30	47	8 (2)	0	(6)	(8)	(6)
decemarticulatum	333 (201)	117 (63)	9	19 (9)	24 (119)	10 (3)	8	9	27 (5)	3	(2)	(9)	(8)
"*latipes*"	609 (215)	192 (67)	31	76 (88)	73 (152)	22 (167)	11	145	34 (17)	0	(19)	(90)	(17)
croxtoni	45 (40)	41 (24)	3	1 (8)	9 (15)	5 (12)	2	0	7 (1)	0	(2)	(16)	(6)
canonicolum	0 (2)	0 (0)	0	0 (7)	0 (0)	0 (0)	0	0	0 (0)	0	(0)	(0)	(0)
Eusimulium "H"	1 (3)	0 (6)	1	15 (203)	0 (1)	0 (0)	0	0	0 (0)	0	(0)	(0)	(0)
Eusimulium n.sp.	0 (0)	0 (2)	0	0 (6)	0 (0)	0 (0)	0	0	0 (0)	0	(0)	(0)	(0)
subexcisum (Edw. of Twinn, 1936)	0 (0)	0 (2)	1	4 (2)	0 (0)	0 (0)	0	0	0 (0)	0	(0)	(0)	(0)
invenusta	1 (3)	0 (0)	0	1 (0)	0 (0)	0 (0)	0	0	0 (0)	0	(0)	(0)	(0)
Cnephia "U"	3 (33)	4 (54)	1	0 (15)	0 (16)	3 (33)	0	1	2 (11)	0	(6)	(0)	(9)
ruggiesi	15 (0)	1 (0)	6	1019 (2150)	0 (4)	0 (0)	1	0	0 (0)	0	(1)	(0)	(0)
venustum	15 (0)	0 (4)	29	5 (1)	1 (0)	0 (0)	1	0	0 (0)	0	(4)	(0)	(0)
decorum	0 (0)	1 (0)	0	0 (0)	0 (0)	0 (0)	0	0	0 (0)	0	(0)	(0)	(0)
Ceratopogonidae													
sphagnumensis	38	878	0	137	0	0	10	0	0	92	—	—	—
stilobezzioides	22	43	0	0	0	1	6	0	0	14	—	—	—
crepuscularis	10	43	0	0	9	0	2	1	1	1	—	—	—
haematopotus	0	9	0	0	3	0	0	0	0	0	—	—	—
obsoletus	0	11	20	0	0	0	0	0	0	0	—	—	—
n.sp. near *piliferus*	0	0	0	Hundreds per collection	0	0	0	0	0	0	—	—	—

NOTE: Collections of *Culicoides* were not kept for identification in 1959.
*1959 results in parentheses.

such avian blood parasites as *Leucocytozoon* (6), *Haemoproteus* (7), and filarioid worms (1). These preliminary observations have shown that some of the biting flies have marked host and habitat preferences. These preferences could conceivably be as important in determining the types of blood parasites found in certain species of birds as the specificity of the parasites themselves.

Miscellaneous Aspects of Feeding Behavior

Generally, most species of biting flies were present in the period June 3–23 (Table VI). Four species, *subexcisum* (Edw. of Twinn, 1936), *Simulium* "H", *Cnephia* "U", and *venustum*, are considered early species; *croxtoni* is considered to be late, although *S. rugglesi* is taken as late as the beginning of August. All species of *Culicoides* first appeared about the same time and all were taken in the greatest numbers during late June and early July.

Most of the *Simulium* (*Eusimulium*) after feeding and leaving the bird, dropped to the floor of the cage and moved slowly to the screen, where they remained relatively quiescent. *S. rugglesi* and *P. decemarticulatum*, on the other hand, tended to fly from the bird to the top of the cage; the former species was particularly active. Some individuals of the woodland species of simuliids remained on the birds for as long as 4 hours, especially if the birds were left undisturbed.

In 1959, a unique opportunity was presented to study some of the habits of the species *Cnephia* "U". Large numbers of this species fed between the hours of 8.30–9.30 p.m. E.S.T.; the sunset time during this period was 8.45–9.00 p.m. E.S.T. It remains to be proved, however, that these black flies were flying after dark. They fed freely on a number of species of birds (Table V) in the forest canopy, but rarely on birds exposed on the forest floor or on the lake shore. The flies took some time in engorging and the bird could be handled

TABLE VI

Biting flies captured on various birds in relation to season, 1958 and 1959

Species	First taken		Abundant
	1958	1959	
Simuliidae			
E. aureum	May 27	May 25	June 4–10
P. decemarticulatum	May 30	May 20	June 4–10
"E. latipes"	May 20	May 24	June 4–10
E. croxtoni	June 4	June 2	June 18–28
E. subexcisum (Edw. of Twinn, 1936)	May 20	May 24	
Eusimulium "H"	May 23	May 24	May 27
Cnephia "U"	May 27	May 24	May 29
S. rugglesi	June 3	June 2	June 11–23
S. venustum	May 23	May 24	May 27
Culicoides			
C. stilobezzioides	June 10	June 7	June 18–30
C. sphagnumensis	June 11	June 7	June 18–30
C. crepuscularis	June 4	June 7	June 21–30
C. haemotopotus	June 9		
C. obsoletus	June 9		
C. nr. *piliferus*	June 9		

quite freely without dislodging the flies, many of which fed about the anus and the heels. The bite of *Cnephia* "U", a large fly, was severe, causing lacerations and haemorrhage at the site which remained inflamed for as long as 3 days. *Cnephia* "U", of all the ornithophilic flies studied, was the only one to produce such gross lesions. Another feature of *Cnephia* "U" was its short period of biting activity (May 24–June 3).

Reports (3) on black flies feeding on mammals suggested that the majority feed between 5.0 and 8.0 p.m. E.S.T. Observations on the ornithophilic forms

TABLE VII

Comparison of flies captured in three 2-hour periods between 1800 and 2400 hours

	1800–2000 hr (168)*	2000–2200 hr (101)	2200–2400 hr (20)
Simulium			
aureum	56	132	2
decemarticulatum	61	169	4
"latipes"	85	321	9
croxtoni	22	29	2
Eusimulium "H"†	12	3	0
subexcisum (Edw. of Twinn, 1936)	5	1	0
rugglesi	687	94	0
venustum	18	2	0
decorum	1	0	0
Cnephia "U"	0	6 (250)‡	25‡
Culicoides			
sphagnumensis	28	688	383
stilobezzioides	11	64	39
crepuscularis	0	23	14
obsoletus	1	27	5
haematopotus	0	2	3
nr. *piliferus*	15	Thousands	Thousands

*Number of collections in parentheses.
†In 1956 and 1957, this species was taken between 1800 and 2000 hours.
‡1959.

TABLE VIII

Summary of data on ornithophilic Simuliidae and Ceratopogonidae

Species	Habitat distribution	Preferred host	Time during evening when feeding	Seasonal abundance
Simuliidae				
**decemarticulatum*	Forest intermediate	Woodland birds	Late	Late May–mid-June
**invenusta*	—	—	—	—
**Cnephia* "U"	Forest intermediate	Woodland birds	Late	Late May
rugglesi	Lake shore	Ducks	Early	June–July
†*venustum*	—	—	—	—
†*decorum*	—	—	—	—
**aureum*	Forest intermediate	Woodland birds	Late	June
**"latipes"*	Forest intermediate	Woodland birds	Late	June
**croxtoni*	Forest intermediate	Woodland birds	Late	Late June
**subexcisum* (Edw. of Twinn, 1936)	Lake shore	Ducks	Early	Late May
**canonicolum*	Lake shore	Ducks	—	Late May
**Eusimulium* "H"	Lake shore	Ducks	Early	Late May
*Unknown *Eusimulium*	Lake shore	Ducks	Early	Late May
Ceratopogonidae				
**sphagnumensis*	Forest intermediate	Woodland birds	Late	June–July
**stilobezzioides*	Forest intermediate	Woodland birds	Late	June–July
†*crepuscularis*	—	—	Late	June–July
haematopotus	—	—	Late	June–July
†*obsoletus*	—	—	Late	June–July
*n.sp. near *piliferus*	Lake shore	Ducks	Late	June–July

*Believed to be the first feeding records.
†Normally feed on mammals.

(Table VII) indicate that all species do not follow this pattern. Most *rugglesi* and *Simulium* "H" were taken between 5.0 and 8.0 p.m. Woodland species, especially *Cnephia* "U", fed in the late evening when the woodland birds are beginning to roost for the night although there is no evidence that any simuliid went on the bird after dark. These and other behavior patterns are summarized in Table VIII.

Feeding by Species of Culicidae and Tabanidae

Various mosquitoes were taken on birds much more frequently in 1957 than in 1958–59, presumably because climatic conditions favored a higher population in the former year. No attempts to catch biting flies at various heights in the forest were made during 1957 and it is impossible to say whether mosquitoes were present at the higher levels during this year of high mosquito numbers. During 1958–59, too few mosquitoes were obtained during each year in both the ground level and forest canopy habitats to permit quantitative conclusions.

Six species (Table IV) were taken in 1957, *Mansonia perturbans* being the most common. During 1958, *Mansonia perturbans* was also quite common. The mosquitoes were taken most frequently on the larger birds.

In both 1957 and 1958, a few specimens of *Chrysops* were taken, after they had fed naturally on birds. In 1957, one specimen of each of *Chrysops inda* O.S. and *Chrysops excitans* Wlk. were obtained, after they had fed on a raven. During 1958, one specimen of each of *Chrysops inda* O.S. and *C. carbonaria* Wlk. feeding on crows were taken. One engorged *Chrysops* sp. was seen leaving a robin tangled in a Japanese mist net. Observations on penned birds, such as ducks, indicated that both *Chrysops* and *Tabanus* were attracted to them. However, the ducks usually ate the flies before they could land and take a meal. In the cases listed above, the birds were either hooded, or unable to move, affording the tabanids an unhampered opportunity for feeding.

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References

1. ANDERSON, R. C. The life cycle and seasonal transmission of *Ornithofilaria fallisensis* Anderson, a parasite of domestic and wild ducks. Can. J. Zool. **34**, 485–525 (1954).
2. BENNETT, G. F. and FALLIS, A. M. Blood parasites of birds in Algonquin Park, Canada, and a discussion of their transmission. Can. J. Zool. **38**, 261–273 (1960).
3. DAVIES, D. M. and PETERSON, B. V. Observations on the mating, feeding, ovarian development, and oviposition of adult black flies (Simuliidae, Diptera). Can. J. Zool. **34**, 615–651 (1956).
4. DOWNES, J. A. Personal communication. 1958.
5. DUNBAR, R. W. The salivary gland chromosomes of seven forms of black flies included in *Eusimulium aureum* Fries. Can. J. Zool. **37**(4), 495–525 (1959).
6. FALLIS, A. M., ANDERSON, R. C., and BENNETT, G. F. Further observations on the transmission and development of *Leucocytozoon simondi*. Can. J. Zool. **34**, 389–404 (1956).
7. FALLIS, A. M. and WOOD, D. M. Biting midges (Diptera:Ceratopogonidae) as intermediate hosts for *Haemoproteus* in ducks. Can. J. Zool. **35**, 424–435 (1957).
8. PETERSON, B. V. Personal communication. 1959.
9. PETERSON, R. T. A field guide to the birds. Houghton Mifflin Co., Boston. 1947.
10. SHEWELL, G. E. Identity of the blackflies that attack ducklings and goslings in Canada (Diptera:Simuliidae). Can. Entomologist, **87**(8), 345–349 (1955).
11. SHEWELL, G. E. Personal communication. 1959.
12. WOOD, D. M. Personal communication. 1958.

THE FAUNA OF ROCKY SHORES OF BARBADOS, WEST INDIES[1]

JOHN B. LEWIS

Abstract

The zonation of the intertidal fauna of the rocky shores of Barbados is described. Although the tidal range is less than 4 ft, the vertical range of the fauna is, in certain situations, much wider. Wave action is shown to be an important factor in extending the vertical range while temperature and exposure influence distribution.

The biology of the common species is described, with emphasis on their reproductive and larval ecology. The majority of the species are copulators and are ovoviviparous or produce egg capsules. There is a marked tendency for the larvae to have a short pelagic life.

Introduction

A recent paper by Maxwell S. Doty (2) has summarized our present knowledge of zonation on rocky intertidal surfaces. He has made it clear that tides, with the varying degrees of exposure they impose, are the primary cause of zonation in the intertidal region. While a basic pattern of zonation is determined by tidal factors, the vertical range of zones may be variously modified by other agencies such as wave action, substrate, etc.

From the point of view of the organisms, living in the intertidal region means varying degrees of adaption to tidal factors. It should be emphasized, however, that this adaption applies to larval stages as well as to adults. The success of any animal in a particular environment is due not only to its ability to maintain itself as an adult but also should be considered as the sum of the successes of the various stages of its life history. The bulk of the literature dealing with the descriptive aspects of intertidal ecology, such as the several papers by T. A. and Anne Stephenson (10, 11, 12), are expressed in terms of the species as adult organisms. The work of Wilson (14) on the settlement of the larvae of marine animals, of Smith (7, 8) on the settlement of barnacle cyprids, and the studies of Kitching (4) and Moore (5) have shown the importance of larval behavior in determining the distribution of adults. Such principles are applicable to the fauna of the intertidal zone.

The purpose of this study, then, is to describe the pattern of vertical distribution of intertidal animals on the rocky shores of Barbados, to discuss the ecology of the more important animals, and to illustrate the adaptions to life on the shore made by larval forms.

General Description

Barbados lies some 90 miles east of the chain of the Lesser Antilles. It has an extreme length of about 21 miles and is 11 miles wide. The long axis is orientated roughly north and south (see Fig. 1). Its total area is 166 sq. miles and its greatest elevation 1104 ft.

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Contribution from the Bellairs Research Institute of McGill University, St. James, Barbados, West Indies.

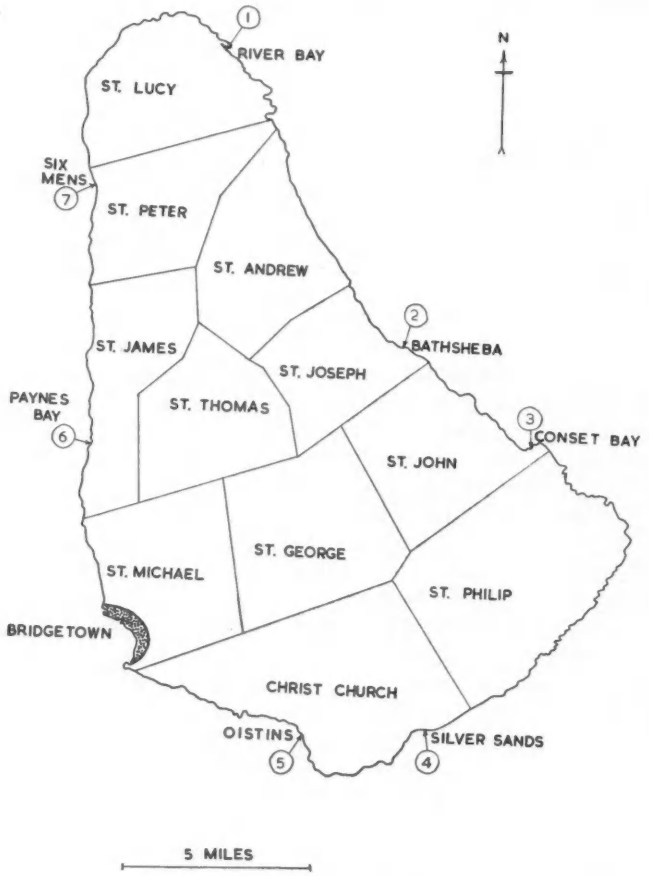

FIG. 1. Map of Barbados showing location of stations.

It is covered for its largest part by Pleistocene reef-limestone (6). This limestone formation rises as a succession of terraces, slopes, and scarps. On the main part of the island, facing north, west, and south, it rises as a series of apparently wave-cut terraces. On the eastern side of the island the limestone has broken away and exposed an underlying oceanic formation of sedimentary rocks. This side of the island shows no rising terraces.

The coast line is regular. There are no deep indentations nor any offshore islands. The length of the coast line is some 60 miles. At the northern end, limestone cliffs rise sharply from the sea to a height of about 100 ft. These extend around the northern shore from Six Men's Bay on the west to a little beyond River Bay on the east.

The western coast has long stretches of sandy beaches, broken by low limestone walls which sometimes rise from the sea and are sometimes set back from the coast.

Along the south coast from Oistins eastward the cliffs again predominate with occasional stretches of sandy beach. These cliffs vary in height from 10 to 15 ft at Oistins and Silver Sands to 100 ft at the southeastern tip of the island.

The eastern coast lies close to the hill region of the island. Much erosion has taken place here in the past. Here and there are sand or boulder beaches, eroded debris in the form of massive limestone boulders and limestone cliffs, some 20 to 25 ft in height.

Environmental Factors

1. Tides

The tides along the coast of Barbados are of the mixed, semidiurnal type, with two highs and two lows each day. There is not a marked inequality between the heights of the tides, however, one tide each day has a greater amplitude than the other. There exists also an inequality in times of each tidal cycle so that there occur periods when there are not two complete cycles in a 24-hour period.

Tidal data have been taken from a U.S. Coast and Geodetic Survey Tide Guage of the standard automatic type. This guage was installed in June of 1957 by personnel of the Lamont Geological Observatory, New York. Over a period of about 18 months the mean tidal range was 2.3 ft and the diurnal range 3.6 ft. The pattern of tide curves for May and October of 1958 is shown in Fig. 2.

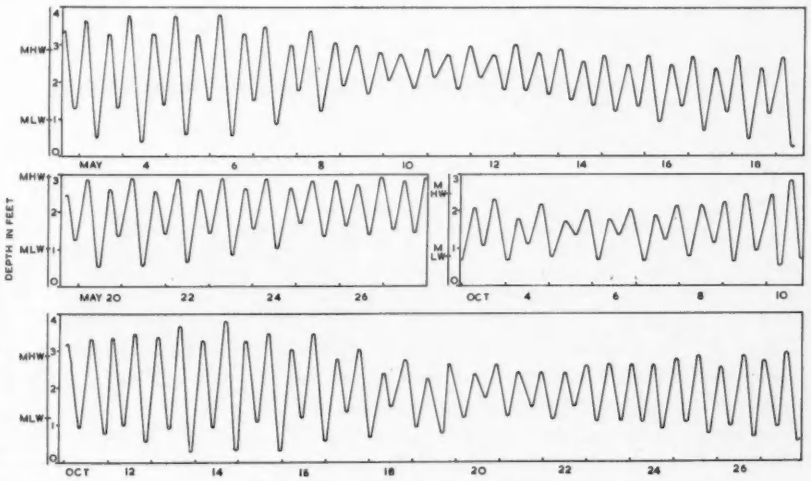

FIG. 2. Tide curves for May and October, 1958.

2. Temperature

The curves of Fig. 3 include the mean monthly air temperatures for Bridgetown, Barbados, the mean monthly sea surface temperatures of the ocean area 10°–15° N. latitude, 55°–60° W. longitude, and the mean monthly sea surface temperatures of inshore waters on the west coast. The air temperatures and sea surface temperatures of the ocean area were taken from the Sailing Directions for the West Indies (15). Sea surface temperatures of coastal waters, made over a period of 1 year, were recorded in shallow water by means of a temperature recorder supplied by the U.S. Office of Naval Research.

The mean monthly air and sea temperatures vary between 25° and 28° C. Highest temperatures were recorded in the summer months and lowest in December and January. The slightly higher temperatures of coastal waters are shown in the figure.

There exists in the shallow coastal waters a slight diurnal variation in temperature. Over a period of 2 weeks, in June of 1957, sea temperatures were recorded at 0800 hours and again at 1300 hours on the same day. There was a mean rise in temperature of 1° C during each 4-hour period.

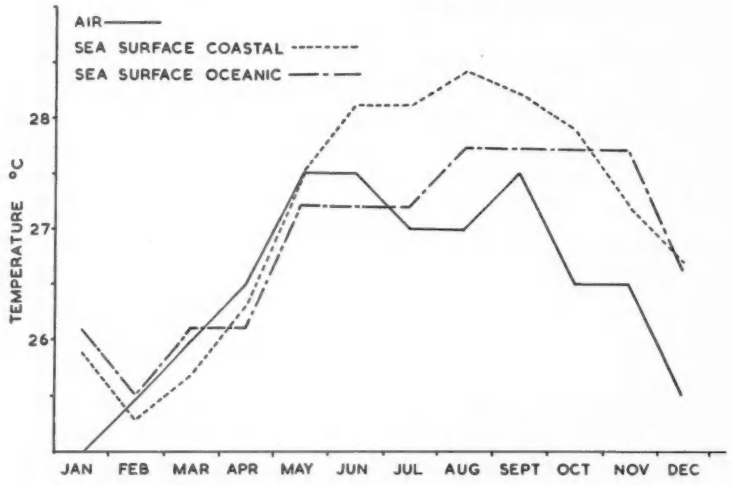

FIG. 3. Mean monthly air and sea surface temperatures.

3. Wave Action

Reproductions of wave records from 3 days in 1957 are shown in Fig. 4. These records were taken from installations of the U.S. Office of Naval Research, placed on the east and west coasts of the island.

Figure 4A shows records of August 25, a day of light winds and low waves. Figure 4B shows records of August 20, on which comparatively moderate wave action occurred around the coast. Figure 4C shows records of October 10, on which heavy wave action was experienced around the island.

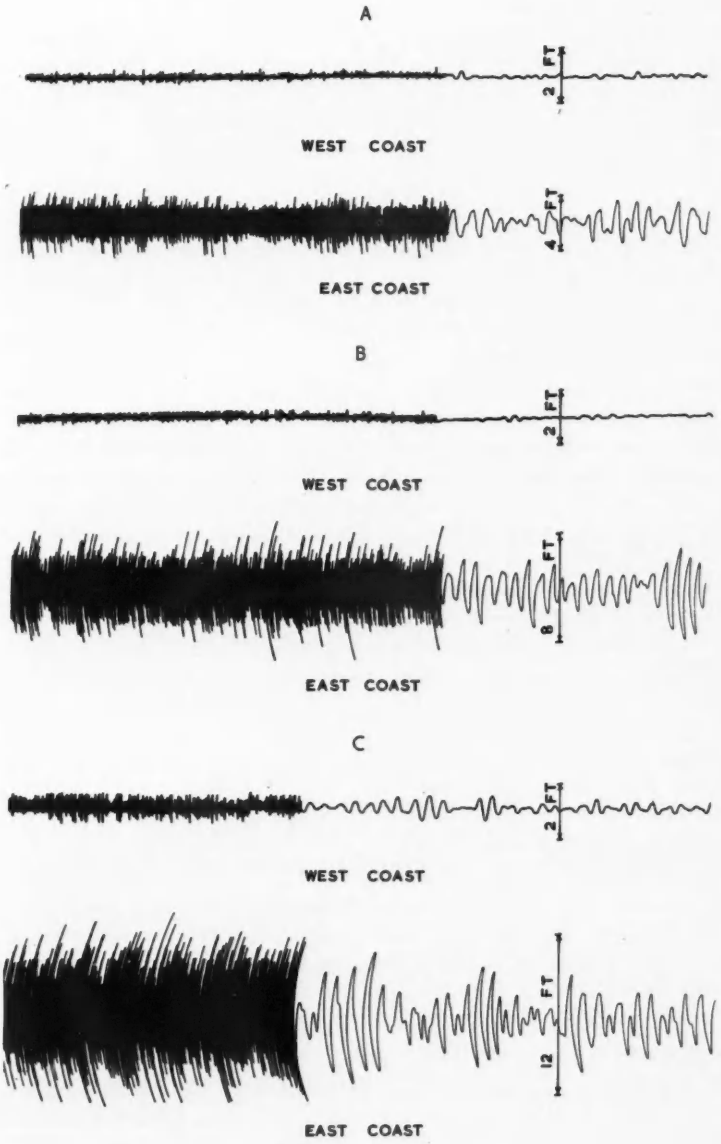

FIG. 4. Surface wave curves, east and west coasts.

There is a marked contrast between the wave amplitudes on each coast on the same days. On a day with low waves the east coast shows waves with amplitude more than four times the amplitude of those on the west coast. On a day with heavy wave action the amplitude of waves on the east coast is greater than eight times those on the west coast. Several years' records obtained by the Office of Naval Research demonstrate that the east coast is continuously subjected to much heavier wave action than is the west coast.

Zonation

Seven stations at widely separated points around the coast of the island were chosen for study. They include the full range of variations in observed environmental conditions. The sites of the stations are shown in Fig. 1. With the exception of Six Men's Bay and Tent Bay, Bathsheba, all stations are limestone cliffs or scarps which are washed by the sea at low or high tides. The scarps vary from 10 to 100 ft in height and are variously modified at their bases by wave action and other erosive agencies.

A typical station is shown in Fig. 5. The face of the scarp is undercut in two places, a little below mean low water mark and at mean high water mark. While the major undercut at high water seems to be due chiefly to wave action, the lower or minor undercutting is probably due to this and to organic destruction as well.

From the low water mark the cliff face rises in a gentle to moderate slope to the inner angle of the major undercut. The return begins from a sharp angle but recedes for a considerable distance so that the undersurface overhangs the bottom. A jutting edge is formed and the cliff face proceeds either vertically upwards as in the case of high scarps or horizontally landwards in the case of low scarps.

Six zones are here recognized. They are termed the *surf zone*, the *pink zone*, the *green zone*, the *black zone*, the *yellow zone*, and the *weather zone*. With the exception of the surf zone they are all visible demarkations of the intertidal region and are clearly delimited by color changes on the surface of the rock.

The measurements of the heights of the zones on the shores were made by means of a taut line stretched from a metal rod imbedded in the rock to the appropriate zone. The metal rod was marked off in 10ths of a ft and the line was levelled with a spirit level. The water level and heights of the zones obtained in this way were referred to the level on the tide gauge pertaining at that particular time.

1. Surf Zone

The surf zone is a relatively narrow band lying from the mean low water mark to approximately mean low water springs. It is a region that was seldom observed completely dry. Even on a day of very light winds the sea swell keeps it intermittently wetted.

The zone is variable in conformation. At Six Men's Bay, Oistins, Silver Sands, and Paynes Bay, it is pitted and has deep fissures and cracks. At

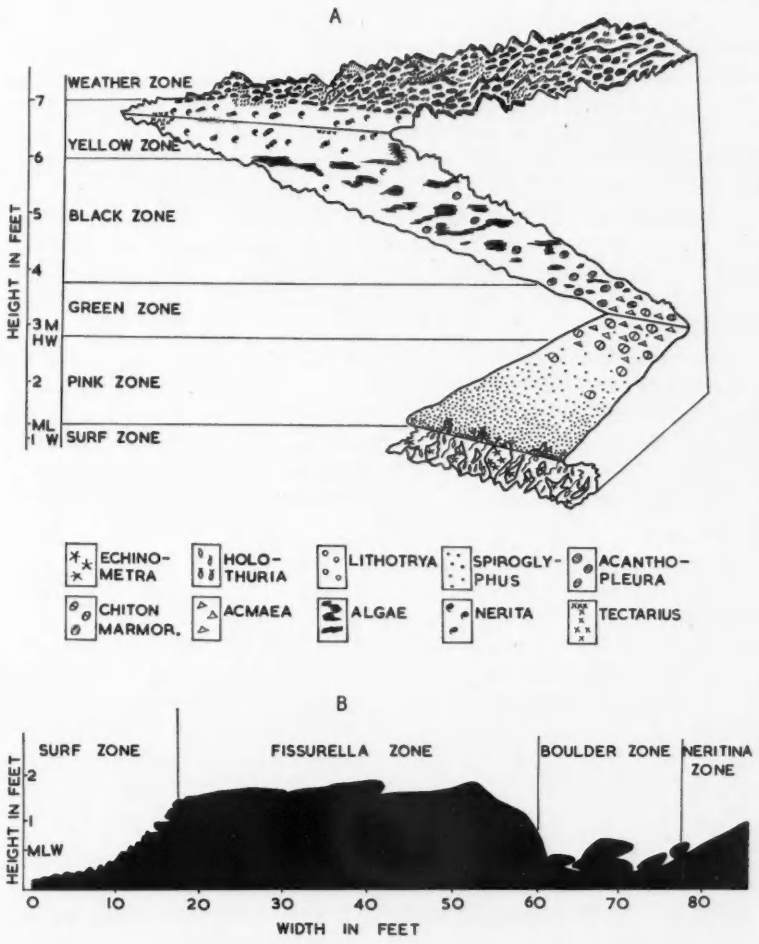

FIG. 5. A. Diagrammatic profile of a typical cliff station. B. Profile of beach platform at Six Men's Bay.

Bathsheba, River Bay, and Conset Bay it consists of the seaward edge of a low, relatively smooth platform fronting the cliff walls. It is often heavily overgrown with algae.

The common organisms found in this zone are as follows: *Echinometra lucunter*, *Fissurella barbadensis*, *Holothuria glaberrima*, *Lithotrya dorsalis*, *Spirobranchus giganteus*, *Bunodosoma cavernata*, sponges, Bryozoa, and *Sargassum* spp. and other algae.

2. Pink Zone

The color of this zone is one of the most prominent features of the rocky shores of Barbados. The pink color is due to the widespread encrustation of coralline algae and is characteristic of all stations. In some places the corallines grow not upon the rock but upon the surface of the colonizing animals themselves. In places the zone is broken and cracked and the pink layer is occasionally overlaid by a leathery black algae which grows in response to a greater degree of wetting. The surface of the zone is usually smooth.

On nearly all shores this zone has a characteristic spatial form. It begins, at its base, at approximately mean low water mark and rises upwards in a gentle arc. The top of the zone is below mean high water mark at most stations. At Bathsheba and Conset Bay the lower limit is included on the frontal platforms.

The most characteristic animal of this zone is the small vermetid *Spiroglyphus irregularis*. This animal occurs at all stations with the exception of Silver Sands where it is replaced by the serpulid *Spirobranchus giganteus*. Other common organisms in this zone are as follows: *Fissurella barbadensis*, *Leucozonia ocellata*, *Thais floridana*, *Bunodactis stelloides*, *Bunodosoma cavernata*, *Bunodosoma kukenthali*, and *Chiton marmoratus*.

3. Green Zone

This zone corresponds approximately to the region of the inner angle of the major undercut of the cliff face. It is pale in color, being usually a light yellow or greenish. This color is due to a thin surface film of microscopic, boring algae which penetrate several millimeters into the rock. The surface of the zone is usually relatively smooth and varies in width from 6 in. at River Bay to nearly 18 in. at Conset Bay. The mean high water mark falls within the limits of this zone at most stations. The common organisms found in the zone are as follows: *Acanthopleura granulata*, *Thais patula*, *Thais floridana*, and *Acmaea jamaicensis*.

4. Black Zone

The black zone is a dominant feature of the shore at all stations. It comprises the upper limb of the major undercut of the cliff face and its upper limit is sharply marked off by the return of the profile. The surface of the rock is very rough and pitted.

The zone is dominated by two species of algae, *Bostrychia tenella* (Vahl) and *Polysiphonia howei* Hollenb. These species flourish away from strong sunlight and when fully developed form a covering which excludes all animals. In most places, however, the growth is patchy. The two species are zoned, *Polysiphonia* lying below *Bostrychia*. The surface of the rock is rough and pitted, hard at some stations, and at others composed of loosely cemented fragments. The common animals of the zone are *Acanthopleura granulata* and *Thais patula*.

5. Yellow Zone

This a zone of yellow-colored rock which corresponds approximately with the outer angle of the major undercut. It is a relatively narrow band usually

less than a foot wide and its surface is very rough. The yellow color of the surface is due to a thin film of algae which has the appearance of being powdered on. The common animals of the zone are *Tectarius tuberculatus*, *Littorina ziczac*, *Nerita peleronta*, and *Nerita versicolor*.

6. Weather Zone

This is the uppermost zone of the intertidal region and it often contains elements of terrestrial origin. On low cliffs land vegetation reaches into this zone. The commonest form is the maritime Convolvulus, *Isopomoea pes-caprae*.

The surface of the zone is extremely rough and dark brown to black in color. This is not due to the rock itself but to the same powderlike film of algae which is found in the yellow zone. This organism darkens when dry and the lighter color of the yellow zone is probably due to wetting by the sea.

There is much boring in this zone as well as in the yellow zone by green and and blue-green algae. Over the whole surface of the zone for some distance above its lower limit is a rind of algae several millimeters thick in the rock.

The common organisms of the zone are *Tectarius tuberculatus*, *Littorina ziczac*, *Nerita peleronta*, and *Nerita versicolor*.

Description of Stations

1. River Bay (Fig. 6)

This station is of special interest because two cliffs, 50–60 ft high, are present near each other but in different positions with respect to the prevailing winds. The bay consists of a long inlet cut out by a river and is oriented parallel to the coast. The west side, while directly exposed to the prevailing winds, is protected to some extent by a low platform running from the base of the cliff for some 20–30 ft into the sea. The east side is subject to virtually no wave action but is subject to rapid fluctuations in water level when, on days of heavy seas, the water rushes into the bay and then flows out again. At the southern tip of the bay these fluctuations are more pronounced than at the northern end. This variation in surge action was observed on several occasions and is evidenced by the amount of undercutting at the base of the cliff. At the southern end, a cavity 3 ft deep has been worn away while at the northern end the cavity is only about 18 in. deep.

The cliffs on both sides are composed of loosely cemented coral and shell fragments. At some places branches of the coral *Acropora* spp. can be pulled easily from the wall.

West Side

The surf zone consists of the outer edge of a platform some 10–15 ft wide, extending seaward from the bottom of the cliff wall. It is just awash at mean low tide and at low water springs is wetted by wave action. It is dominated by low growths of algae of which a *Sargassum* spp. is the principal form.

The pink zone is composed of the inner edge of the platform and the base of the scarp. The surface is smooth and bright pink in color. The dominant

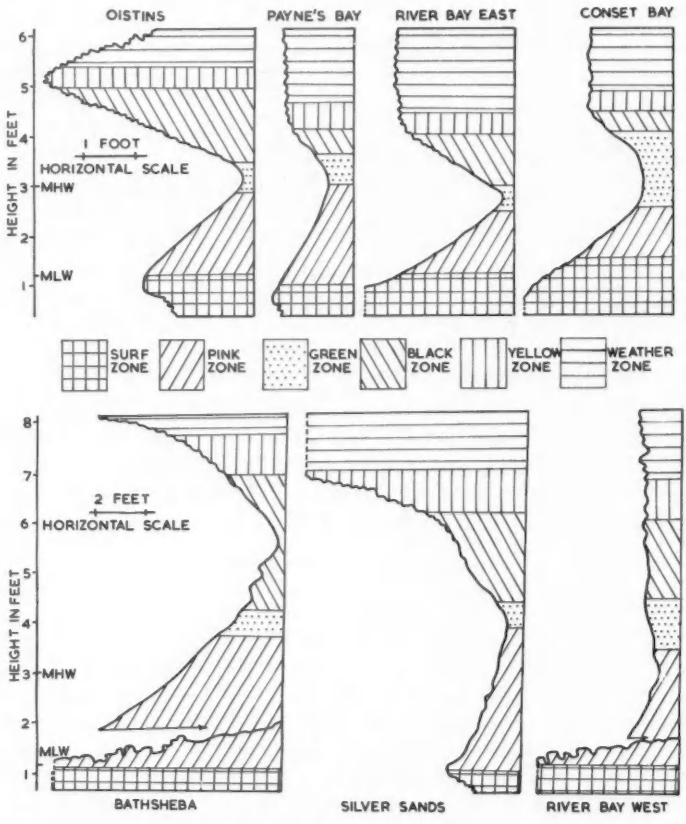

FIG. 6. Profiles of cliff stations showing zonation.

animal of this zone is the small vermetid *Spiroglyphus*. Along the outer edge of the zone, in potholes and crevasses, *Echinometra lucunter* occurs abundantly, and closely associated with it are *Bunodosoma cavernata* and *Bunodosoma kukenthali*. The latter is more abundant than the former. Imbedded deep in the rock with its oral face covered with sand grains is the actinian *Bunodactis stelloides*. *Fissurella barbadensis* is found here but is more abundant on the inner edge of the zone. The hermit crabs *Clibanarius tricolor* and *Calcinus tibicen* and the calico crab, *Eriphia gonogra*, are also abundant. *Holothuria glaberrima* is abundant in sheltered crevasses, and the boring barnacle *Lithotrya dorsalis* around the edges of potholes.

On the inner half of the pink zone, which includes a portion of the platform as well as the cliff face, are found *Spiroglyphus irregularis*, *Fissurella barbadensis*, *Thais floridana*, *Thais patula*, *Bunodactis stelloides*, *Holothuria glaberrima*, and the small *Nerita tesselata*. In holes on the platform are found

small cling fish and the occasional small colony of the coral *Siderastrea radians*. The rock-boring barnacle *Lithotrya dorsalis* is common on any vertical faces.

The upper level of the pink zone is a little above mean high water mark.

The green zone is a narrow band of smooth, bared rock about 12 in. wide. A few small vermetids are present but the common animals of this zone are *Acanthopleura granulata*, *Thais patula*, *Thais floridana*, and *Nerita tessellata*.

The surfaces of the black, yellow, and weather zones are extremely rough and sharp. The algae *Bostrychia* and *polysiphonia* occur frequently in the black zone together with *Acanthopleura granulata* and *Thais patula*. *Tectarius tuberculatus*, *Littorina ziczac*, *Nerita peleronta*, and *N. versicolor* occur above in the yellow and weather zones. Beneath many of the shells of *N. peleronta* were found the small isopod *Ligia gracilis* Moore. The lower limit of the weather zone lies about 4 ft above mean high water mark.

East Side

The surf zone on the east side of River Bay consists of a platform some few feet in width. It is submerged about half way up at mean low water. It supports a vigorous growth of several species of algae. A *Sargassum* spp. is dominant at the southern end. The common animals of the zone are *Echinometra lucunter*, *Leucozonia ocellata*, *Thais floridana*, *Livona pica*, and *Holothuria glaberrima*.

The rock surface is smooth in the pink zone but is not as much colored by coralline algae as at some other stations. *Spiroglyphus irregularis* is the dominant form. *Chiton marmoratus* is abundant and *Fissurella barbadensis*, *Thais floridana*, and *Leucozonia ocellata* are common. *Spirobranchus giganteus* occurs in small colonies. The upper limit for the zone lies about 6 in. below mean high water mark.

The green zone lies in the angle of the major undercutting of the scarp wall. On the lower limb the rock is smooth and worn, while above, it is composed of loosely cemented coral fragments with many fissures and holes. *Chiton marmoratus* occurs on the lower limb and *Acanthopleura granulata* above. *Thais floridana*, *T. patula*, and *Leucozonia ocellata* are present in small numbers.

The surface of the rock is very rough in the black, yellow, and weather zones. There is a sparse growth of the algae *Bostrychia* and *Polysiphonia* in the black zone along with *Acanthopleura granulata* and *Thais patula*. The yellow zone is a narrow band just at the return of the cliff face. The weather zone extends for several feet above it. The common animals of the two zones are *Tectarius tuberculatus*, *Littorina ziczac*, *Nerita peleronta*, and *N. versicolor*.

2. Bathsheba (Fig. 6)

The coast line at this station is rather irregular. The scarp has been much eroded, leaving immense boulders with areas of sandy beach between. These boulders and the remains of the scarp are considerably undercut from wave action for they face directly into the prevailing winds. At high water the waves break close to the cliff over a long frontal platform which extends for a distance of some 100 ft from the scarp. At low water the inner portion of this platform is exposed and offers protection from the waves to the wall.

At the site selected for the station a portion of the coast which has resisted erosion projects into the sea. It is protected on both sides and in front by a platform. At low water the latter is just bare and at high water is submerged and the waves break directly upon the cliff face.

The surf zone lies on the frontal platform at a distance of 20–30 ft from the base of the cliff. A heavy growth of algae, principally a *Sargassum* spp., covers the whole zone. *Thais floridana*, *Leucozonia ocellata*, and *Fissurella barbadensis* occur in this zone but are not at all abundant.

The pink zone is a wide one which includes about 20 ft of the frontal platform and the basal portion of the wall. It has a smooth but irregular surface with many potholes and crevasses. *Spiroglyphus irregularis* is the dominant animal and completely covers large portions of the rock surface. In the potholes are *Echinometra lucunter* and *Bunodosoma kukenthali*. The zooanthid *Palythoa variabilis* is common here in shallow depressions which hold water at low tide or upon surfaces that lie beneath the shadow of the cliff overhang. The sabellarid *Phragmatopoma californica* is common at the base of the cliff where it forms a crust about an inch thick. It flourishes best in sites sheltered from sun and surf.

Around the lips of the depressions and potholes of the pink zone, the rock is perforated by the holes of the boring barnacle *Lithotrya dorsalis*. *Chiton marmoratus*, *Bunodactis stelloides*, and a few *Phymanthus crucifer* deeply imbedded in the substrate are present. The upper limit of the zone lies some 6–8 in. above mean high water mark.

The green zone corresponds roughly to the inner angle of the major undercut. It is a narrow zone, no more than 6 in. wide at most. *Acmaea jamaicensis*, *Acanthopleura granulata*, and *Spirobranchus giganteus* are present.

There is a moderate growth of *Bostrychia* and *Polysiphonia* together with *Acanthopleura granulata*, *Thais patula*, and a few *Nerita peleronta* in the black zone.

The yellow zone is nearly a foot wide but is irregular and nonexistent in places. *Littorina ziczac*, *Tectarius tuberculatus*, *Nerita peleronta*, and *N. versicolor* are present in both yellow and weather zones.

Tent Bay, Bathsheba

Tent Bay station is about $\frac{1}{4}$ of a mile south of Bathsheba station. It is of interest because it is one of the few pebble beaches on the island. The beach is about 150 yd long and is limited at either end by projections of the scarp reaching the sea.

Rounded boulders, a few inches to a foot or two in diameter, of sandstone, conglomerate, and shale overlie a sand bottom. From the beach, a reef flat, just awash at low tide, leads for some few yards out to sea. Boulders and outcrops of limestone are present along the outer edge of the beach.

At high water the beach is completely covered with water and there is a continuous shifting and rolling of the smaller boulders. Only near the low water mark is there any stability, for it is here that the largest boulders are aligned.

The fauna of the beach is restricted to a narrow band about 6 ft wide alongshore. The spaces between the boulders are awash at mean low tide and the sand bottom beneath remains saturated. The tops of the boulders are intermittently wetted.

Beneath the boulders and pebbles is to be found a distinct fauna. *Chiton tuberculatus*, *Holothuria glaberrima*, *Tegula excavata*, *Bunodosoma cavernata*, *Bunodactis stelloides*, *Planaxis nucleus*, *P. lineatus*, *Nitidella ocellata*, *N. laevigata*, and a small reddish goby (*Gobiesox* spp.) are all common. On top of the boulders are *Bunodosoma kukenthali*, *Nerita tessellata*, *Thais floridana*, and the hermit crab *Clibanarius tricolor*.

Behind the beach is a limestone wall, partly composed of country rock and partly of blocks laid down for the bed of a former railway. Here are to be found the only large colonies of *Melampus coffeus*, feeding on decayed vegetation. *Tectarius tuberculatus*, *Tectarius muricatus*, and *Nerita versicolor* are also common here.

3. Conset Bay (Fig. 6)

The coast at Conset Bay consists of limestone cliffs between 60 and 100 ft high. Some 50 yards off shore there is a rubble bank, bare at mean low water, which offers protection to the shore. On the inner edge of this bank there is a narrow channel and from this a platform rises up and continues shoreward to meet the cliff. At high tide both the platform and the bank are covered by some 3 ft of water.

The surf zone at this station lies upon the low platform and is 20–30 ft in width. It is dominated by an abundant growth of algae. A *Sargassum* species is dominant along the outer edge.

The pink zone begins near the base of the cliffs at mean low water mark. As at other stations it is covered by coralline algae which overlays the dominant animal, *Spiroglyphus irregularis*. The other common animals in the zone are *Leucozonia ocellata*, *Thais floridana*, and *Fissurella barbadensis*. The latter also extends its range into the surf zone. In crevasses and potholes at the base of the pink zone and extending into the surf zone are found *Echinometra lucunter*, *Holothuria glaberrima*, *Lithotrya dorsalis*, *Bunodosoma kukenthali*, and *Nerita tessellata*. The green zooanthid *Zooanthus pulchellus* forms extensive colonies in places on the vertical face.

Acmaea jamaicensis is the dominant animal in the green zone. Also common are *Fissurella barbadensis*, *Chiton marmoratus*, *Acanthopleura granulata*, and *Thais floridana*. A few individuals of the sessile barnacle *Tetraclita squamosa* are present. The green zone is about 18 in. wide at this station and its lower limit lies 6–8 in. below mean high water mark.

The rock surface above the green zone is extremely rough and pitted. There are heavy growths of the algae *Bostrychia* and *Polysiphonia* in the black zone together with *Acanthopleura granulata* and *Thais patula*. *Tectarius tuberculatus*, *Littorina ziczac*, and the two *Neritas*, *N. peleronta* and *N. versicolor*, are found above in the yellow and weather zones.

4. Silver Sands (Fig. 6)

Silver Sands is an extensive area of sand dunes and bright beach. At the eastern end, where the station was chosen, an outcrop of limestone forms a low rampart which rises up from the sea to an elevation of 10–15 ft. There is an alongshore current and the area is subjected to heavy wave action, especially during the winter months.

There is a marked undercutting of the shore, and the cliff face projects out over the sea for a distance of up to 15 ft. There is a rocky bottom composed mostly of cemented limestone debris which is covered by a foot of water at low tide.

The surf zone is strongly undercut and is extremely irregular. The surface is broken by deep cracks, fissures, and rounded holes. In nearly all the holes are groups of the urchin *Echinometra lucunter*. Also abundant are the holothurian *Holothuria glaberrima* and the anemone *Bunodosoma kukenthali*. The zone is very colorful for there is a wide variety of encrusting sponges, rounded colonies of a *Lithothamnion*, groups of a *Sargassum* spp., and encrusting white Bryozoa.

The pink zone is subdivided at this station. On the lower half, at mean low water level, the serpulid *Spirobranchus giganteus* is dominant and has settled as a complete covering over the substrate. On the upper half of the zone the small vermetid *Spiroglyphus irregularis* has assumed dominance. *Chiton marmoratus* is abundant in the upper half and *Thais floridana*, *T. patula*, and *Leucozonia ocellata* are present. *Echinometra lucunter* extends upwards wherever there are suitable cracks and holes. The upper limit of the zone lies nearly a foot above mean high water mark.

The green zone is rather narrow at this station and is dominated by the limpet *Acmaea jamaicensis*. A few vermetids are present along the lower edge and *Acanthopleura granulata* along the upper.

Acanthopleura granulata and *Thais patula* are present in the black zone between patches of the algae *Bostrychia*. *Littorina ziczac* and *Tectarius tuberculatus* are common in the yellow and weather zones, the latter being extremely abundant in places. *Nerita peleronta* is present but in small numbers and the same applies to *N. versicolor*.

5. Oistins (Fig. 6)

This station is similar to the one at Silver Sands. It has limestone cliffs 8–10 ft high but there is less pronounced undercutting than at Silver Sands.

The surf zone comprises the minor undercut at the base of the scarp and is raised 6 in. to a foot above a sandy bottom. It receives the impact of even low waves and is only intermittently bare, never dry. The surface is smooth. Pink coralline algae and a leathery, black encrusting algae intermingle with large colonies of *Spirobranchus giganteus*. The more pronounced the undercutting the heavier the population of *Spirobranchus*. Also abundant in this zone are *Holothuria glaberrima*, *Bunodosoma kukenthali*, and *Lithotrya dorsalis*. *Echinometra lucunter* is present but is not common.

Spiroglyphus irregularis is dominant in the pink zone and is overlaid by coralline algae. *Chiton marmoratus* is abundant and there are small colonies of *Spirobranchus giganteus*. At the bottom of the zone, in a line almost exactly dividing it from the surf zone, are tufts of the coarse, green filamentous algae *Enteromorpha lingulata*. The upper limit of the pink zone lies just at mean high water mark.

The green zone is dominated by the limpet *Acmaea jamaicensis*. A few vermetids, *Spirobranchus giganteus* and *Chiton marmoratus* extend upwards from the zone below and *Acanthopleura granulata* and *Thais patula* from the zone above.

The algae *Bostrychia* and *Polysiphonia* are not abundant at this station. *Acanthopleura granulata* is abundant in the black zone and *Thais patula*, common. A few *Tectarius tuberculatus* and *Littorina ziczac* occur in the black zone.

The surface of the rock is extremely rough in the yellow and weather zones. *Nerita peleronta* and *N. versicolor* are common and *Tectarius tuberculatus* is extremely abundant.

6. Paynes Bay (Fig. 6)

This is the only cliff station on the western coast of the island. It consists of a limestone wall some 10–20 ft in height which rises directly from the sea. The cliff is only a little undercut in this area and it is only during the winter months that this coast is subject to heavy seas.

The surf zone is nearly vertical or only slightly undercut. It has small cracks and fissures in which are found the urchin *Echinometra lucunter*. *Spirobranchus giganteus* is the only other common form in this zone.

The pink zone is covered by a layer of coralline algae which in places is overlaid by a black algae which encrusts like a leathery integument. *Spiroglyphus irregularis* and *Acmaea jamaicensis* are codominants. There are small colonies of the green zooanthids *Zooanthus pulchellus* and *Spirobranchus giganteus*. *Fissurella barbadensis* is common and there are a few individuals of the barnacle *Tetraclita squamosa*.

The green zone is bright in color with a light-yellow cast. The surface is rough with many holes and cracks. *Acmaea jamaicensis* is the dominant form with a few *Acanthopleura granulata* present.

The black zone is not sharply marked off from the green. Patches of *Polysiphonia* extend into the green zone, and *Acmaea jamaicensis* reaches up into the black. *Acanthopleura granulata* and *Thais patula* are the common animals. The surface is relatively smooth.

The yellow and weather zones are very rough and sharp. *Littorina ziczac*, *Tectariis tuberculatus*, and *Nerita versicolor* are common but not abundant and *N. peleronta* is scarce.

7. Six Men's Bay (Fig. 5B)

This station consists of a low-lying platform of beach rock of cemented limestone fragments and sand grains. It rises from a sand bottom at a slope of

45 degrees to about 18 in. above mean low water mark and continues with only a slight rise for a distance of 75 ft to a sand beach. The outer edge is deeply fissured and has a very irregular surface with many cracks and holes. The horizontal surface is for the most part quite smooth.

Four zones are recognized at this station: a *surf zone* which corresponds roughly to the surf zones of cliff stations, a *Fissurella zone* which is dominated by the limpet *Fissurella barbadensis* and has affinities with the pink zone, *a boulder zone*, and a *Neritina–Ulva zone*.

The surf zone is deeply marked by fissures which run shorewards from the outer edge. *Echinometra lucunter* is the dominant animal. It lives in excavated holes up to the top of the platform. Closely associated with this urchin are a small fish, a porcellanid crab, and a limpet. *Thais deltoidea* is common here, and there are several species of crabs. There is a thin and irregular veneer of coralline algae over the rock surface.

The Fissurella zone is an almost horizontal platform, smooth on its forepart and roughened behind, with several cracks running parallel to the shore. At the south end of the platform there is a heavy encrustation of *Zooanthus pulchellus*. At the north end, portions of the zone have a heavy matted covering of low algae and here *Fissurella barbadensis* thrives best.

Shoreward of the Fissurella–Zooanthus region is a ledge with a longitudinal crack in which water is held at low tide, in a band about 2 ft wide. Juveniles of the urchin *Tripneustes esculentus* and of *Diadema antillarum* collect here. Under the lip of the ledge is the boring barnacle *Lithotrya dorsalis*, the pelecypod *Isognomon listeri*, a variety of crabs, and large numbers of boring worms.

Behind this crack is a region of smooth bared rock which supports a heavy population of *Tetraclita squamosa*. Also abundant here are *Spiroglyphus irregularis*, *Petaloconchus* (cf. *varians*), *Nerita tessellata*, *Acmaea jamaicensis*, and *Thais floridana*.

The boulder zone is a low-lying area which is filled with water at low tide. There are openings to the sea at either end so that water is not retained for any length of time. The tops of the boulders reach to a height of about 1 ft above mean low water. On the sides of the boulders are *Fissurella barbadensis*, *Acmaea jamaicensis*, and *Nerita tessellata*. On the undersides of the boulders, dry at low water, is a dense population of *Spirobranchus giganteus*. The hermit crabs *Clibanarius tricolor* and *Calcinus tibicen* abound around the bases of the boulders.

The Neritina–Ulva zone is about 15 ft wide and terminates in a sandy beach. The line of the beach fluctuates so that at times the zone may be partly covered with sand. It supports a heavy growth of *Ulva lactuca* when uncovered. *Neritina pupa* and the two species of hermit crabs are abundant in this zone. *Littorina maleagris* and *Nerita tessellata* are common.

Comparison of Stations and Influence of Physical Factors

The profiles of Fig. 6 show a marked difference between stations of the vertical positions of the various zones. The vertical distance between the top of the surf zone, which corresponds approximately to the mean low water

mark in each station, to the bottom of the weather zone varies between 3 ft and nearly 7 ft. Considering that the extreme tide range for the island is not quite 4 ft it is evident that other factors besides tides are responsible for the observed zonation on the shores.

Four stations, Oistins, Paynes Bay, River Bay (east), and Conset Bay, show similar zonation patterns and ranges. At each of these stations the mean low water mark is equal to the lower limit of the pink zone. Mean high water mark is the upper limit of this zone at Oistins and is approximately so at the other three stations. This mark also corresponds closely with the inner angle of the major undercut of the cliff profile. At Oistins, Paynes Bay, and River Bay (east), the green zone is a narrow band about 8 in. wide and includes the angle of the cliff profile. At Conset Bay the green zone is about 18 in. wide but here too it comprises the greater part of the angle.

The black zone varies from 18 in. wide at Oistins to less than 12 in. wide at River Bay (east), and less than 6 in. at Conset Bay. At both Oistins and River Bay the angles of the cliff profiles are acute and deep while at Paynes Bay and Conset Bay the angles are less pronounced. At all four stations the upper limit of the black zone is marked by the return of the cliff profile. While the upper limit in each case is not equidistant from mean low water mark it is at the same relative position of the profile.

The yellow zone in each case is a narrow band about 6 in. wide and is succeeded at each station by the weather zone.

These four stations are situated on the southwest, west, northeast, and east coasts (see Fig. 1). Paynes Bay on the west and Oistins on the southwest are not in the line of the prevailing winds and will not be subject to as heavy wave action as the east coast (see Fig. 4). While the other two stations, River Bay (east) and Conset Bay, are in the line of the prevailing winds, both have special features which protect them from wave action. Thus all four stations are subject to only moderate wave action normally.

At Bathsheba, Silver Sands, and River Bay (west), the total vertical range of the zones is much wider than at the four stations considered above. In all three the lower limit of the pink zone is at mean low water mark. At Bathsheba and River Bay (west) there are long frontal platforms so that this zone occupies a considerable area. The upper limit of this zone is about 6 in. above mean high water mark. It is followed by the green zone which is a narrow band from 6 in. to a foot wide at each station. The center of this zone lies about $3\frac{1}{2}$ ft above mean low water.

Above the green zone lies the black zone, a band which is about 18 in. wide at River Bay west and nearly 3 ft wide at Bathsheba. The succeeding zone, the yellow, is about a foot wide in each case and at Bathsheba and Silver Sands lies just below the lip of the cliff profile.

The geographical positions of these three stations are as follows: Bathsheba and River Bay are on the east and northeast coasts respectively and Silver Sands is on the southeast. Thus all three are exposed to the prevailing winds. The curves of Fig. 4 demonstrate the heavy wave action experienced on the east coast during normal weather.

Comparing the two sets of stations we find that the group on the east and south coasts have wider vertical zones than do those on the west and that the east and south group are subjected to heavier wave action. River Bay (east) which has the narrowest zone spectrum is subject to the least wave action of all stations. Bathsheba has the widest zone spectrum and is subject to the heaviest wave action.

There is evidence that temperature exerts an influence on the pattern of zonation in certain cases. The serpulid worm *Spirobranchus giganteus* occurs normally in the surf zone and lower part of the pink zone. However, in several places its vertical range is extended to lie well above mean low water. At Six Men's Bay it is abundant under the overhangs of large irregular boulders and at River Bay and Bathsheba there are large colonies in small caves. The same is true to a lesser degree of the sabellarid *Phragmatopoma californica*. The best-developed colonies are found in caves at River Bay and Bathsheba.

The two species of algae which characterize the black zone, *Polysiphonia* and *Bostrychia*, are best developed on cliffs with a deep overhang and are poorly developed in places such as Paynes Bay where there is no overhang.

These distributions suggest that sites shaded from direct sunlight are preferred by these four organisms. The microclimate of intertidal shores at Plymouth has been described by Southward (9), who has demonstrated the heating of exposed rock surfaces and that animals may have their body temperatures raised by exposure to the sun. Certainly it was evident from casual observation that rock surfaces exposed to the sun quickly became hot to the touch.

Biology of the Commonly Occurring Species

Fissurella barbadensis Gmelin (Fig. 7)

Barbados is the type locality for this common West Indian limpet. It is found at all stations but is most abundant upon the platforms of beach rock such as occur at Six Men's Bay. On vertical shores it occurs upon the frontal platforms and only to a limited extent upon the lower portion of the cliff walls. It occupies a low position on the shore, in the surf zone, and about half way up into the pink zone or from just below mean low water mark to mean sea level.

Fissurella breeds throughout most of the year. Ripe individuals were taken from March to November. The eggs are a light green in color and are 0.16 mm in diameter. They are enclosed within large round capsules about 0.2 mm in diameter. A fertilized egg develops to the four-celled stage within an hour of fertilization and within 12 hours is motile within its capsule. The capsule ruptures at this stage and a bright-green trochophore larva, 0.18 mm in diameter, is released. Within 24 hours of fertilization the larval shell develops.

The larva is about 0.2 mm in diameter. The body is light green in color and the larval shell is transparent with a light network of fine lines in a diamond-shaped pattern. The larvae are active swimmers at first but in the laboratory many of them begin to crawl on the bottom of the container

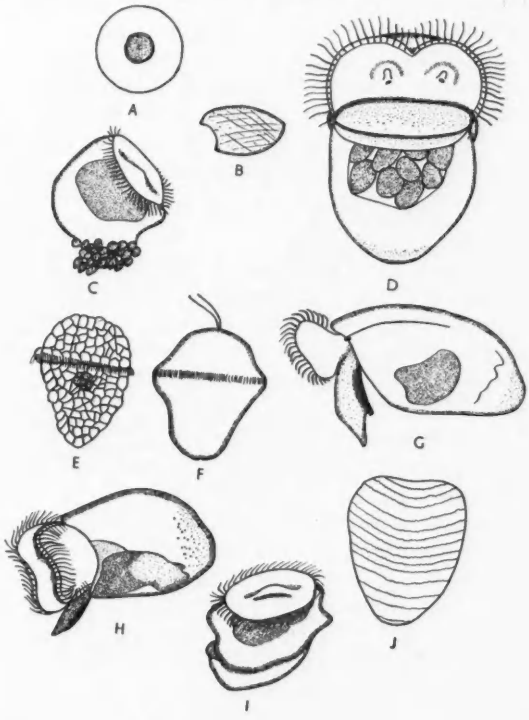

FIG. 7. A. Egg of *Fissurella*. B. Larval shell of *Fissurella*. C. Trochophore larva of *Fissurella*. D. Swimming larva of *Fissurella*. E and F. Early larvae of *Acmaea*. G. Swimming larva of *Acmaea*. H. Swimming larva of *Hemitoma*. I. Trochophore larva of *Hemitoma*. J. Larval shell of *Acmaea*.

within a day or two. The pelagic life is thus a short one, lasting only 2 or 3 days.

The shell of adults of *Fissurella* is variable in shape and color. Many individuals are completely coated with a pink covering of a coralline algae while others have heavy growths of filamentous green algae. Small individuals tend to be found near low water mark together with large specimens with a low shell apex. Large-sized adults found higher in the intertidal region are regular in form with perfectly crenulated edges to the shells and have high ribs and high shell apexes.

Acmaea jamaicensis Gmelin (Fig. 7)

This little limpet is common at all cliff stations with the exception of River Bay. It is often a dominant form in the green zone from about mean tide level to above high water mark. Its upper limit is extended at stations which are exposed to heavy wave action.

Breeding individuals were taken from March through November. Trochophore larvae are released within a few hours of fertilization. They are light pink in color and about 0.12 mm long. Within 24 hours of fertilization the larval shell develops. The newly formed larva is an active swimmer at first but within a day or two begins to crawl about on the bottom of the container. The species has, then, a pelagic life of only 2 or 3 days. The larvae are light pink in color and the shell is yellow with characteristic transverse markings. They are about 0.2 mm in length.

Hemitoma octoradiata (Gmelin) (Fig. 7)

Only at one station, Six Men's Bay, is this colorful little limpet at all abundant. It occurs in the surf zone up to a little above mean low water mark. It is found in holes and crevasses in the rock and is nearly always associated with the urchin *Echinometra lucunter*. It lives beneath the test of the urchin, firmly attached to the bottom of the depression or hole inhabited by the echinoid.

The shell shape of the species is variable. It is an almost flat disk in younger specimens and only develops ridges and a high apex in old specimens which live in a sheltered site.

Breeding individuals were found in August and September only. The eggs are light brown in color and are 0.12 mm in diameter. A shelled larva is released within 12 hours of fertilization. The trochophore stage is apparently passed within the egg capsule. The shelled larva is a light green in color with a dark-brown central body and a transparent shell. They are about 0.24 mm in length and are active swimmers upon hatching. No crawling larvae were observed in the laboratory after a period of several days. This would indicate that the species may have a slightly longer larval life than either *Fissurella* or *Acmaea*.

Acanthopleura granulata Gmelin (Fig. 8)

This species, perhaps the commonest of West Indian chitons, is common at all stations in Barbados. It is found in the green and black zones, from mean sea level to some distance above mean high water mark.

Acanthopleura breeds in the autumn months from October to December. The peculiar eggs which are shed freely into the water are light brown in color and are 0.32 mm in diameter. They are covered with a dense layer of short stout spines and when laid form a lightly compacted mass, held together by the interlocking spines.

The larvae are released from the capsule within 24 hours of fertilization. They are 0.2 mm in length and are active swimmers. They are pear-shaped with a median fringe of long cilia and four long apical flagella. In the laboratory, larvae lost their swimming powers within a day or two and settled down on the bottom of the glass finger bowls in which they were reared. They metamorphosed slowly, passing through a crawling stage shown in Fig. 8C. In this stage they are about 0.24 mm in length. It is a bilobed form with the smaller segment bearing the apical flagella. These flagella are lost within a few hours of settling as is the fringe of cilia. A pair of bright red eyespots

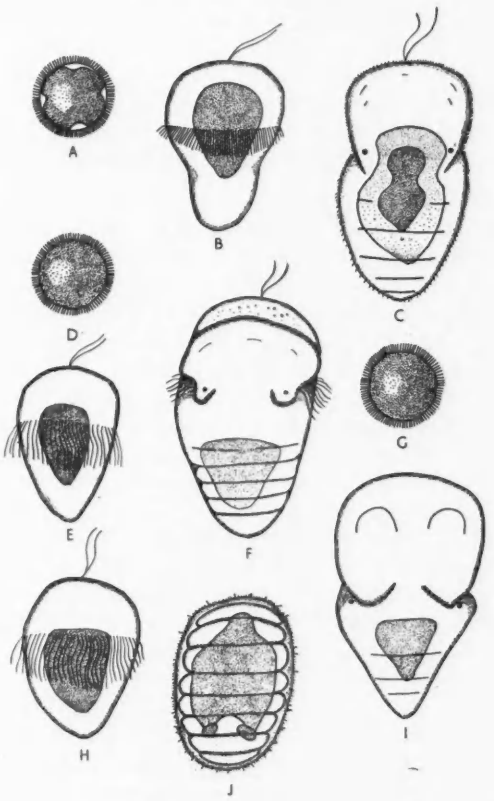

FIG. 8. A. Egg of *Acanthopleura*. B. Trochophore larva of *Acanthopleura*. C. Settling larva of *Acanthopleura*. D. Egg of *Chiton marmoratus*. E. Trochophore larva of *Chiton marmoratus*. F. Settling larva of *Chiton marmoratus*. G. Egg of *Chiton tuberculatus*. H. Trochophore larva of *Chiton tuberculatus*. I. Settling larva of *Chiton tuberculatus*. J. Juvenile of *Chiton tuberculatus*.

develop at this time. The larva is a light green in color. Within a day the adult plates begin to develop and metamorphosis is complete within 4–5 days of fertilization. The pelagic life is thus a short one.

Chiton marmoratus Gmelin (Fig. 8)

This chiton is common at all cliff stations and is abundant at River Bay and at Bathsheba. It is characteristic of the pink zone and the lower portion of the green zone. It is a rather more active species than *Acanthopleura*.

Acanthopleura

Breeding occurs from October to about the end of November. The eggs are similar to those of *Acanthopleura* in appearance. They are 0.20 mm in diameter and light green in color. Swimming larvae are released within 12

hours of the fertilizing of the eggs and an intermediate crawling stage develops within 2 days. The crawling stages are similar to those of *Acanthopleura*, losing their flagella and fringe of cilia, developing a waist, and crawling about on the bottom of the container. There is a pair of red eyespots and several scattered red pigment spots. The juvenile plates begin to develop soon after settling and the pelagic stage of the species is short.

Chiton tuberculatus Linnaeus (Fig. 8)

This handsome chiton occurs occasionally at all cliff stations but is abundant at Tent Bay, Bathsheba. It occurs in the region of mean low water to mean sea level, but only in situations in which it can remain unexposed to the air. At Tent Bay an abundant population occurs on the undersides of stones on the boulder beach. Here it is found up to mean sea level and above but always beneath a rock where there is a reservoir of moisture. At cliff stations it is found in the surf zone beneath the minor undercut of the cliff face.

The species tends to be gregarious, groups of five or six usually occurring together. It shows a strong negatively phototropic reaction and glides swiftly away from light on an overturned rock. It is the most active of the three species of chitons.

Breeding takes place from October to December. The light-green eggs with their covering of spines are 0.24 mm in diameter. Larvae are released within 24 hours of the fertilizing of the eggs. The actively swimming larva is somewhat rounder than that of *C. marmoratus* but resembles the latter in other respects. It is a dark brown in color with a light-green patch and several small red pigment spots.

The pelagic life of the species is short. Metamorphosis begins about 3 days after fertilization. The settling larva retains its fringe of cilia for a day and then begins to show traces of plate formation. The crawling larvae are sticky to the touch and adhere strongly to the bottom of the container.

Metamorphosis was completed within 5 days in the laboratory. The juvenile chiton shown in Fig. 8J is 0.28 mm in length. The eight plates are completely formed and there is a girdle of short irregular spines.

Thais patula Linnaeus (Fig. 9)

This, the largest of the three *Thais* species occurring in Barbados, is common at all stations. It is most abundant in the black zone and green zone, from mean sea level to a foot or two above mean high water.

It is apparently a nocturnal species, living in the daytime in depressions and cracks in the rock and in the laboratory it rests in darkened corners. It feeds chiefly upon the chiton *Acanthopleura granulata* and upon the barnacle *Tetraclita squamosa*.

Breeding occurs in the late summer from August through September. Fifty to 100 small white eggs, 0.24 mm in diameter, are laid in egg capsules below low water mark, usually beneath flat stones. The capsules are about 4 mm in diameter and are laid in closely packed clusters of up to 200. They are flat and rounded, slightly opaque, and with a small clear central region through which the larvae escape.

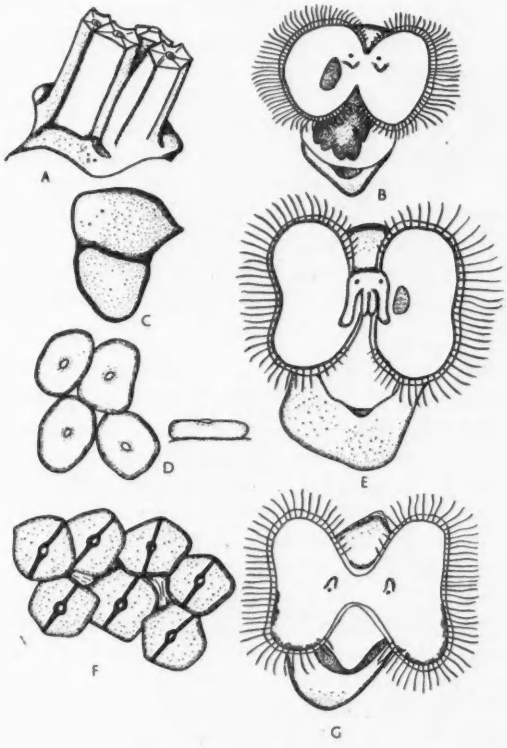

FIG. 9. A. Egg capsules of *Thais floridana*. B. Veliger of *Thais floridana*. C. Shell of veliger of *Thais patula*. D. Egg capsules of *Thais patula*. E. Veliger of *Thais patula*. F. Egg capsules of *Thais deltoidea*. G. Veliger of *Thais deltoidea*.

Development in the capsule takes about 2 weeks. Freshly hatched larvae are 0.4 mm in length. The larval shell is dark brown in color and pitted with small dots. The velar lobes of the larvae have distinctive dark-brown stripes around the edges and a round white body appears through the region of the left lobe. The shell has a distinctive shape, with a wide aperture and the outer lip pointed to the left and slightly grooved to form a short canal.

The larvae are active swimmers at hatching. Laboratory-reared specimens lived for over a week without settling and the species must be presumed to have a fairly long pelagic life.

Thais floridana Conrad (Fig. 9)

T. floridana is common at all stations on the east coast. It is a lower-living species than *T. patula* and is found in the pink zone from below mean low water to about mean sea level.

The species breeds during the early summer, from the end of March to early June. The egg capsules are attached to the undersides of rocks below

mean low water. They are 3.2 mm in height, hexagonal in section, and about 1.6 mm in diameter. They are creamy white in color. Several hundred eggs are laid in each capsule.

Swimming larvae hatch within 12 to 14 days after the eggs are laid. They are 0.16 mm in diameter. The larval shell is light brown in color and there is a dark-red pigment body in the region of the right velar lobe. Like the larvae of *T. patula* the larvae of this species remained as swimming larvae in the laboratory for up to a week without change and the species is thus presumed to have a fairly long pelagic life.

T. floridana is carnivorous and feeds upon barnacles, chitons, and other molluscs. Larger specimens are often completely covered with a thick layer of coralline algae.

Thais deltoidea Lamarck (Fig. 9)

This is the least common of the three *Thais* species found in Barbados. It is nowhere abundant and is common only upon the beach rock platform at Six Men's Bay and upon the frontal platform at Bathsheba. It also occurs commonly at Six Men's below low water level on a rocky bottom. It is a low level species, found in the surf zone and lower portion of the pink zone.

The species breeds in October and November, later than the other two species. Egg capsules are laid in patches in holes and depressions in the rock below low water. They are round and flattened and can be distinguished from those of *T. patula* by the presence of a narrow dark band which bisects the capsule. They are laid in groups of 40–50 with the median bands orientated in the same direction.

The freshly hatched larvae are 0.36 mm long. The shell is a dark brown in color and is pitted with small dots. There is a light golden stripe around the circumference of the velar lobes and the aperture of the shell is pointed. Laboratory-reared specimens lived for only a few days but there is no reason to suppose that the pelagic life of this species is any shorter than that of the other two *Thais* species.

Tectarius tuberculatus Wood (Fig. 10)

This species is a very common form at nearly all stations and in places is exceedingly abundant. It is most abundant on vertical walls where the surface is rough and deeply pitted. It remains within hollows during the day and moves about at night.

It is a characteristic form of the weather zone but occasionally young specimens are found as low as the high water mark. Larger specimens are usually found highest in the vertical range.

The species breeds throughout the year. The eggs are laid singly in beehive-shaped capsules or in small capsules deposited in long strings. The egg size is 0.08 mm and that of the capsule 0.25 mm. A swimming larva is released within 3 days of the laying of the egg. The larva is 0.12 mm long and is a light brown in color. It bears a pair of large eyespots on the velum and a dark, heavily pigmented body situated near the apex of the shell. It is an active

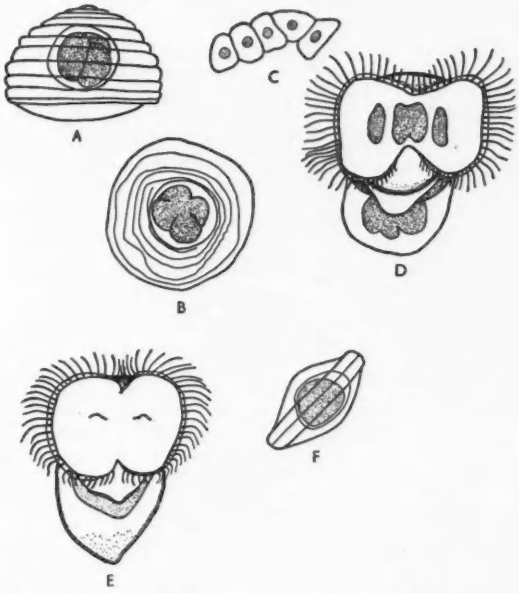

FIG. 10. A and B. Egg capsules of *Tectarius tuberculatus*. C. String capsules of *Tectarius tuberculatus*. D. Veliger of *Tectarius tuberculatus*. E. Veliger of *Tectarius muricatus*. F. Egg capsule of *Tectarius muricatus*.

swimmer. Of the larvae reared in the laboratory none appeared ready to settle after 1 week and the species is presumed to have a fairly long pelagic life.

Tectarius muricatus Linnaeus (Fig. 10)

This species is common at only two stations, at Bathsheba and at Conset Bay. It was taken only occasionally at the other stations. It occurs well up in the weather zone on rocky outcrops and is often found amongst land vegetation.

It breeds during the latter part of the summer, from about mid-June until the end of August. Eggs are laid singly within a small flattened transparent capsule. The egg diameter is 0.08 mm and that of the capsule 0.32 mm. The egg develops within 24 hours to a small swimming larva which breaks through the capsule. The larva is 0.1 mm in length.

Littorina ziczac Gmelin (Fig. 11)

L. ziczac is abundant at all cliff stations. It occurs in the weather zone chiefly but is also found upon isolated rocks as low as mean sea level. It lives in depressions in the rock, and small specimens are usually found near the lower limit of its vertical range.

Breeding occurs throughout the year. Eggs are laid singly in small capsules. Two types of capsules were found. The smaller of the two is 0.24 mm in diameter and is shaped like a beehive. It is by far the commoner. The second type is

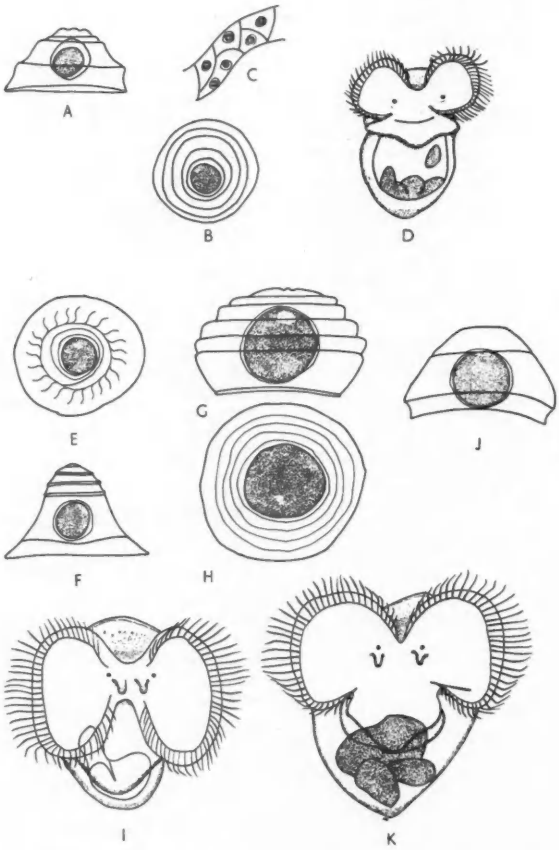

FIG. 11. A and B. Egg capsule of *Littorina maleagris*. C. String capsules of *Littorina maleagris*. D. Veliger of *Littorina maleagris*. E and F. Large type capsules of *Littorina ziczac*. G and H. Small type egg capsules of *Littorina ziczac*. I. Veliger of *Littorina ziczac*. J. Egg capsule of *Neritina pupa*. K. Veliger of *Neritina pupa*.

0.72 mm in diameter and is helmet-shaped. The egg diameters of the two types are 0.08 mm and 0.16 mm respectively.

Swimming larvae develop within 12 hours of the laying of the eggs. The larval shell, 0.28 mm long, is a light yellow in color with a red rim around the lip of the aperture. A prominent dark-green body shows clearly through the shell. As larvae were kept in the laboratory for periods up to a week without settling, the species is presumed to have a fairly long pelagic life.

Littorina maleagris Potiez and Michaud (Fig. 11)

This little *Littorina* is not a common form in Barbados and was found only at Six Men's Bay and Paynes Bay on the inner edge of beach rock platforms.

In 1954 it was very abundant at Six Men's in a narrow belt just below high water mark but the species has since become less abundant there.

The eggs are laid in small transparent capsules, either singly or in strings, in November and December. The egg diameter is 0.08 mm and that of the capsules 0.24 mm. The larvae hatch in about 3 days after the eggs are laid. They are light brown in color, are active swimmers, and are 0.12 mm in length.

Neritina pupa Linnaeus (Fig. 11)

This pretty little shell is found commonly only at Six Men's Bay and on one other area on a sea wall near Bridgetown. It is abundant at both places. It lives near high water mark on the platform at Six Men's Bay. It is a highly gregarious animal, large aggregations being found together in hollows in the rock which hold a little water or remain damp at low tide. The portion of the platform on which it dwells is often subject to heavy scouring by sand but sustains periodically heavy growths of the algae *Ulva lactuca*.

There is considerable variation in the color pattern of this species; smaller specimens tend to be almost completely black while older individuals have a bright pattern of black and white. The lip of the shell is often broken and rough and larger specimens often have the whole shell deeply pitted and eroded.

Eggs are laid singly in capsules in July and August. The egg diameter is 0.08 mm and the beehive-shaped capsule 0.25 mm. Larvae are shed within 2 days after the eggs are laid and the larvae are active swimmers. They are a light brown in color with a conspicuous dark-brown gut and are 0.13 mm in length.

Conus mus Hwass (Fig. 12)

Conus mus is common at stations on the east and south coasts but is nowhere abundant. It is not strictly an intertidal form but occurs in pools at low water and beneath flat stones exposed at low tide. It is not at all tolerant to drying out. It breeds during the summer from mid-April to August. Copulating pairs were observed in groups of up to a dozen under rocks at low water level. The eggs are laid in horny capsules, several hundred eggs to a capsule. The caspules are deposited in rows with their bases attached firmly to the rock. They are about 5 mm long and are flattened and white in color. The eggs are 0.12 mm in diameter. They hatch within 10 days of laying and distinctive swimming larvae are released. The larval shell is 0.24 mm long, light yellow in color, and with a red rim around the lip of the shell. There is a conspicuous ring of lemon-yellow pigment dots around the outer edge of the velar lobes and a pair of dark-pigmented bodies which show through the shell.

Larvae reared in the laboratory lived for a few days only. However, because of its distinctive yellow markings the species was recognized in plankton hauls. By virtue of the large size of the planktonic larvae it is assumed that the species has a fairly long pelagic life.

Leucozonia ocellata Gmelin (Fig. 12)

Leucozonia is found at all stations and is common at River Bay and at Bathsheba. It is characteristic of the pink zone from about mean sea level to low water mark or a little below. It is commonly found in crevasses or in the open on rock surfaces on which there is a heavy algal growth or a heavy population of vermetids.

The species breeds from February to the end of May or June. Several hundred trumpet-shaped capsules are laid by aggregations of copulating pairs beneath stones or on the surface of the rock just below mean low water. The egg capsules are about 4 mm in height and are attached by a stalk to the substrate. They are light pink in color and each contains several dozen eggs.

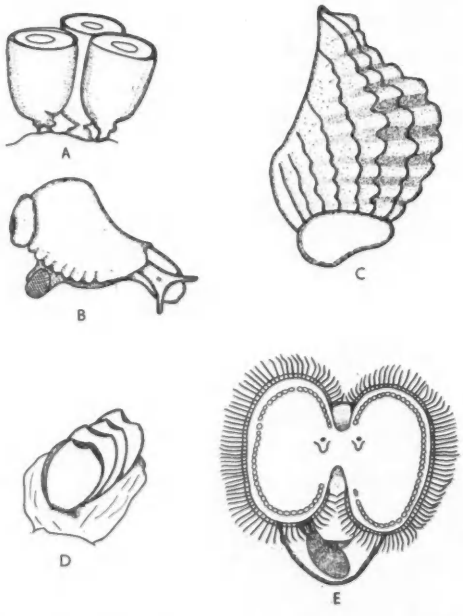

FIG. 12. A. Egg capsules of *Leucozonia ocellata*. B. Larva of *Leucozonia ocellata*. C. Shell of larva of *Leucozonia ocellata*. D. Egg capsules of *Conus mus*. E. Veliger of *Conus mus*.

There is no pelagic stage in this species. The larvae hatch as crawling individuals and adhere strongly to the bottom of the container. The shell of the larva at hatching is 1.8 mm long, light pink in color, and with a scalloped edge and well-defined ridges.

Adults were observed feeding upon vermetids, barnacles, and small anemones.

Astrea caelata Gmelin (Fig. 13)

This species is not at all abundant in Barbados. A small number of specimens of moderate size occur at all stations and they are fairly common at Bathsheba. They occur low in the intertidal zone, up to about mean low water mark. They live beneath flat rocks or in crevasses in the surf zone.

Astrea breeds during June and July and ripe individuals occur in small breeding groups. The eggs are bright green in color, 0.20 mm in diameter, and are laid in a jellylike matrix which adheres lightly to the bottom of the container. The eggs hatch to trochophore larvae within 12 hours of the fertilizing of the egg. The larva is a light green in color with a darker central body and is 0.16 mm in diameter. It is an active swimmer. Larvae were kept for 6 days in the laboratory without further development. It is thus assumed that the species has a long pelagic life.

Livona pica Linnaeus

Livona pica is not at all abundant in Barbados but occurs in small numbers at all stations. It is most common on the east coast at Bathsheba and Conset

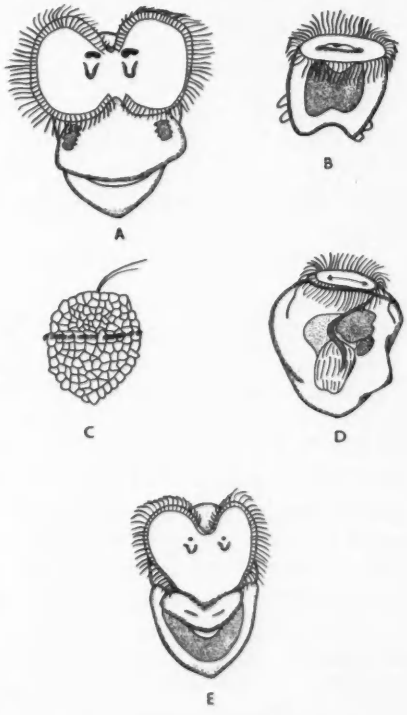

FIG. 13. A. Veliger of *Petaloconchus* cf. *varians*. B. Trochophore of *Astrea caelata*. C. Trochophore of *Isognomon listeri*. D. Veliger of *Isognomon listeri*. E. Veliger of *Planaxis nucleus*.

Bay. It is found in the surf zone, and small specimens live in the pink zone above mean low water on the exposed frontal platforms at Bathsheba. It is most common in the cracks and fissures of the surf zone and under rocks below low water mark.

No breeding individuals of this species were collected.

Planaxis nucleus Lamarck (Fig. 13)

This species is abundant at River Bay and at Bathsheba. It occurs occasionally at all other stations. At Bathsheba it is found on the pebble beach at Tent Bay. It occurs from low water to about mean sea level, depending upon the amount of splash, and the upper level varies from day to day. The species is abundant beneath and around the edges of the stones which comprise the beach and is found upon the upper surfaces if the splash is heavy.

Breeding occurs from the end of April to the end of June. The species is ovoviviparous. From 50 to 100 larvae are released by each female. The larvae are 0.12 mm in length, transparent with a light-brown shell, and are active swimmers. Larvae kept in the laboratory for more than a week showed no signs of being ready to settle and the species is thus presumed to have a fairly long pelagic life.

Planaxis lineatus Da Costa

This tiny shell occurs abundantly at Six Men's Bay, Bathsheba, and Conset Bay. It dwells beneath flat rocks in the region of mean low water. Several hundred specimens often occur beneath a single flat rock.

Breeding occurs from May to the end of June and like *P. nucleus* this species is ovoviviparous.

The color of the adults varies from dark brown to pure white.

Nerita peleronta Linnaeus (Fig. 14)

This is the largest of the *Nerita* species occurring in Barbados. It is found at all stations and is abundant at all stations but Six Men's Bay. It is found in the yellow and weather zones, well above high water mark. It occurs occasionally down into the black zone and it lives firmly attached to the cliff walls in depressions or is hidden within deep crevasses.

Breeding takes place during the summer. The eggs are laid in white, helmet-shaped capsules attached firmly to the rock in groups of a dozen or more. These capsules are about 2 mm in diameter and are never laid high up in the weather zone but rather near the lower edge and into the black zone. The eggs hatch in about 2 weeks and an actively swimming veliger breaks through the capsule. The larvae are 0.45 mm long. They possess a light-brown shell and there is a pair of darkly pigmented kidney-shaped bodies which show clearly through the shell.

No development beyond the pelagic stage was observed in laboratory-reared specimens kept for over a week. The species is thus presumed to have a fairly long pelagic life.

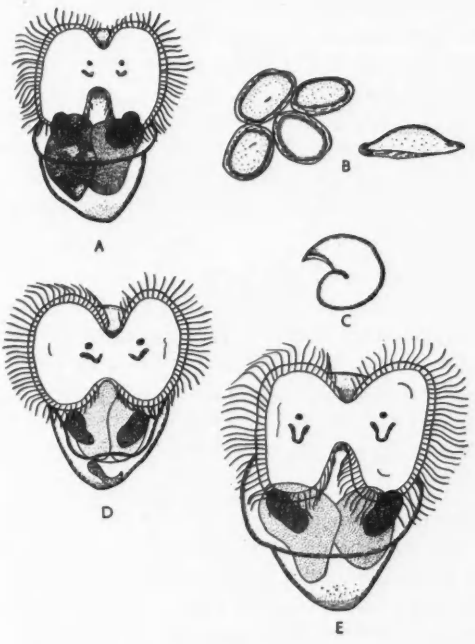

FIG. 14. A. Veliger of *Nerita tessellata*. B. Egg capsules of *Nerita tessellata*. C. Shell of veliger of *Nerita tessellata*. D. Veliger of *Nerita versicolor*. E. Veliger of *Nerita peleronta*.

Nerita versicolor Gmelin (Fig. 14)

Like *N. peleronta* this species is found at all stations and occurs above high water mark in the black and weather zones.

Breeding takes place in June, July, and August. The egg capsules are about 2 mm wide and are laid on the surface of the rock a little above mean sea level. The veliger larvae are similar to those of *N. peleronta* but are distinguishable from the latter by their smaller size. They are 0.35 mm long. They are active swimmers and like *N. peleronta* apparently have a fairly long pelagic life.

Nerita tessellata Gmelin (Fig. 14)

This is by far the most abundant of the three *Nerita* species found in Barbados. It occurs at all stations but has a different vertical distribution from the other two species. It is seldom found on a cliff face but prefers the tops of loose rocks bared at low tide and the underside of flat rocks above mean low water mark. It shows a preference for shaded areas. As many as a hundred are often found together under flat rocks at about mean sea level.

It is common at Six Men's Bay where it lives upon the platform that is completely exposed at mean low tide. Here it is subject to direct sunlight.

The egg capsules of this species have been observed throughout the year. The eggs are laid in shallow depressions which usually hold water at low tide and on the undersides of rocks just below mean low water mark. The capsules are about 1 mm in diameter.

The veliger larvae hatch about 2 weeks after the eggs are deposited. They are similar in appearance to the larvae of the other two species but are only 0.2 mm in length. They are active swimmers and lived for more than a week in the laboratory without settling. The species is thus presumed to have a fairly long pelagic life.

Tegula excavata Lamarck

This species occurs abundantly at Tent Bay, Bathsheba. It is found in small numbers only at other stations. It lives beneath the rocks of the boulder beach at Tent Bay, from mean low water mark to about mean sea level. Although this beach is subject to considerable surf action at high tide, *Tegula* remains protected beneath the cover of the boulders.

Breeding occurs in December and January. The eggs and sperm are shed freely into the water.

Columbella mercatoria (Linnaeus)

This species was taken only at River Bay and at Bathsheba. It is not abundant at either station. It is found beneath flat rocks in shallow water below mean low water mark. Several specimens are usually found together.

Large numbers of dead shells, inhabited by hermit crabs, were observed at Six Men's Bay and at River Bay. It is thus likely that the species is more abundant below the intertidal region.

No breeding individuals were collected.

Melampus coffeus Linne

This is a rare species in Barbados. A colony of several hundred individuals was found at Tent Bay. Here it dwells well above high water mark in sheltered crevasses on a limestone wall. It was observed feeding upon rotting vegetation.

No breeding individuals were collected.

Spiroglyphus irregularis (Orbigny) (Fig. 15)

This species is abundant at all stations. It is a characteristic form of the pink zone and has a vertical range from mean low water mark to nearly mean high water. It is one of the most successful animals on the shore and is nearly always the dominant form in the pink zone. In some places colonies completely cover the rock and exclude the settlement of any other sessile animals or plants.

Breeding occurs throughout the year. The species is partly ovoviviparous. The larvae are released singly or developing eggs are shed into the water in a capsule. Each capsule contains several to a dozen eggs, all at the same stage of development. The developmental stages vary between capsules however.

Both swimming and crawling larvae are released by the female. The swimming larva is 0.36 mm in length and has a white shell which darkens within

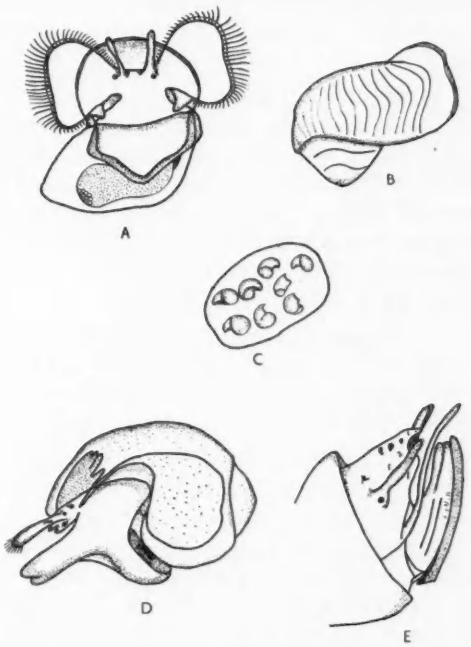

FIG. 15. A. Veliger of *Spiroglyphus irregularis*. B. Shell of crawling larva of *Spiroglyphus irregularis*. C. Egg capsule of *Spiroglyphus irregularis*. D. Crawling larva of *Spiroglyphus irregularis*. E. Head region of crawling larva of *Spiroglyphus irregularis*.

a few hours. The swimming larva settles within a few hours to a day or two and immediately becomes firmly cemented to the substrate. The crawling larva is similar to the pelagic stage but lacks the velum and has a well-developed foot. Upon settling both stages become firmly attached and within a day are impossible to dislodge without breaking the shell.

The adults feed by ejecting strings of mucous from the mouth and then withdrawing the strings, with the entrapped food particles, back into the mouth. Feeding activity was more prevalent when the animals were subjected to a current of water than when the water was not agitated.

Spiroglyphus thus has a very short pelagic life as a veliger but egg capsules may be released and the eggs take up to 2 weeks to develop.

Petaloconchus cf. *varians* (Orbigny) (Fig. 13)

This species occurs occasionally at all stations but is common only at Six Men's Bay. It lives upon the rocky platform between mean low tide and mean sea level. It is a larger species than *Spiroglyphus*, reaching a length of over 25 cm.

The species is ovoviviparous but unlike *Spiroglyphus* the larvae are entirely pelagic. The larvae are about 0.2 mm long and have a light-brown shell. A

pair of dark-brown bodies show clearly through the shell. The larvae are active swimmers and were kept in the laboratory for over a week without settling. They are thus presumed to have a fairly long pelagic life.

Nitidella laevigata Linne

N. laevigata is found in small numbers at Six Men's Bay and is quite common at the east coast stations: Conset Bay, Bathsheba, and River Bay. It occurs under flat stones in shallow water at about mean low water mark. It is a gregarious species and several dozen are usually found together. It is most abundant at Tent Bay on the boulder beach.

No breeding individuals were collected.

Nitidella ocellata Gmelin

This handsome little species has the same distribution as *N. laevigata*. It is found under rocks at about mean low water mark at Tent Bay, River Bay, and Conset Bay. An albinistic form of the species occurs in about half of the population. Both color forms are found living together.

No breeding individuals were collected.

Drupa nodulosa C. B. Adams

This little shell is quite common at River Bay, Bathsheba, and Conset Bay. It lives beneath flat rocks up to about mean sea level and also upon the cliff walls in crevasses in the pink zone. It occurs at Bathsheba upon the platform fronting the cliff wall. The shell is often broken at the apex and discolored by coralline algae and other organisms.

No breeding individuals were collected.

Isognomon listeri (Hanley) (Fig. 13)

Isognomon is fairly common at all stations. It is not restricted to the intertidal zone but is also found in depths of several feet under flat rocks and stones. In the intertidal zone it is found up to about mean sea level at Six Men's Bay and just above mean low water at cliff stations.

It lives deep within cracks and fissures in the rock or under flat stones which are stable and hold moisture. It is never found upon the surface of the rock where it would be subjected to full exposure at low tide. It is extremely variable in shape and conforms to the shape of the cavity which it occupies.

Breeding occurs in September, October, and November. The eggs are shed freely into the water and hatch within 4 hours of fertilization. A simple trochophore larva is released and develops into a veliger in about 4 days. The species is presumed to have a fairly long pelagic life.

Lithotrya dorsalis Sowerby

This rock-boring barnacle is very common and abundant at all stations. It is found chiefly in the surf zone but reaches a little way up into the pink zone. The vertical distribution is thus a narrow belt extending from a little above mean low water to below extreme low water.

Lithotrya has a short breeding season. In June and July pairs of flattened ovaries produce up to a few hundred eggs each. The larvae are typical cyprids.

A freshly hatched cyprid has a bright-red pigment spot and is 0.32 mm in length. A 12-hour cyprid is just double this length.

Tetraclita squamosa stalactifera (Lamarck)

This barnacle occurs occasionally at all stations but is only abundant on platforms such as exist at Six Men's Bay and at Bathsheba. It does not seem to be present on any sea walls around the island but vigorous populations occur on wharf pilings in several areas.

Tetraclita occurs at low levels of the intertidal region, from mean low water to about mean sea level. Measurements of its vertical distribution on pilings showed a range of about 1 ft between mean low water and mean sea level. On rocky platforms the species prefers ridges and high points and is seldom found in hollows or depressions.

Breeding occurs throughout the year. Ripe individuals were found in every month, and newly settled specimens appeared continuously upon pilings.

Grapsus grapsus (Linnaeus) (Fig. 16)

This widely distributed crab is common at all stations. It occurs throughout the intertidal region and to some distance above high water.

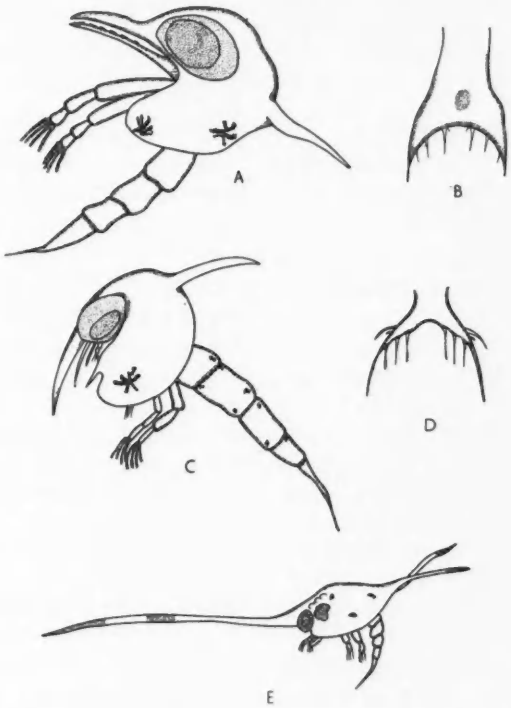

FIG. 16. A. Larva of *Grapsus grapsus*. B. Telson of same. C. Larva of *Eriphia gonogra*. D. Telson of same. E. Larva of *Petrolisthes vanderhorsti*.

Grapsus breeds in early spring in Barbados, from February through May or June. Ovigerous females carrying their dark-brown eggs appeared as late as August however.

The larvae when shed into the water are strongly positively phototropic. They are relatively large zoeae, 2.1 mm in length. They have a light-brown body with a red patch on the telson. The eyes are large and there are two dark pigment granules upon the carapace.

Petrolisthes vanderhorsti Schmitt (Fig. 16)

This attractive little porcellanid crab which reaches a size of about 1 cm is dark maroon in color, marked with bright-blue spots. It lives beneath flat rocks in shallow water below low water and in the intertidal region it is found almost exclusively beneath the test of *Echinometra lucunter* in the surf zone.

Breeding takes place in March and April. Each female carries about a dozen to 20 bright-orange eggs. Freshly hatched larvae are 1.6 mm in length, half of which is the length of the rostral spine. The carapace is marked with sparse red pigment spots, the two posterior spines are tipped with yellow, and the rostral spine has two bright-yellow bands.

Eriphia gonogra (Fabricius) (Fig. 16)

Eriphia, the calico crab, is one of the commonest crabs of the intertidal region. It is abundant at all stations. It occurs from below mean low water to nearly the top of the pink zone, wherever there are holes and fissures for it to hide in.

It breeds from March to June. Up to 50 or 60 dark-brown eggs are carried by a ripe female. The larvae are about 2 mm long at hatching and are a light brown in color with scattered darker pigment patches. They are strongly positively phototropic.

Clibanarius tricolor (Gibbes) (Fig. 17)

Clibanarius is a handsome little hermit crab which is abundant at all stations except those which have a vertical cliff face which rises directly from the sea. It is extremely abundant at River Bay where there are extensive shallow water flats. Hundreds of shells containing the crabs are gathered together at this station under flat rocks.

It is a very small form and inhabits a wide variety of shells. It occurs chiefly under stones below mean low water and in sheltered crevasses up to mean tide level. At Six Men's Bay it occurs among algae on the platform, fully exposed to the sun.

Breeding takes place from April to June. About 20 bright-orange eggs are carried by a ripe female. The larvae are peculiar in that they do not have the typical bent zoeae form. They are 1.6 mm in length, have bright-blue eyes, a brown body with reddish markings upon the thorax, abdomen, and rostrum.

Calcinus tibicen (Herbst) (Fig. 17)

This species is similar to *Clibanarius* in distribution but is nowhere as abundant. It is a larger species and is easily distinguished by its bright-red chelae.

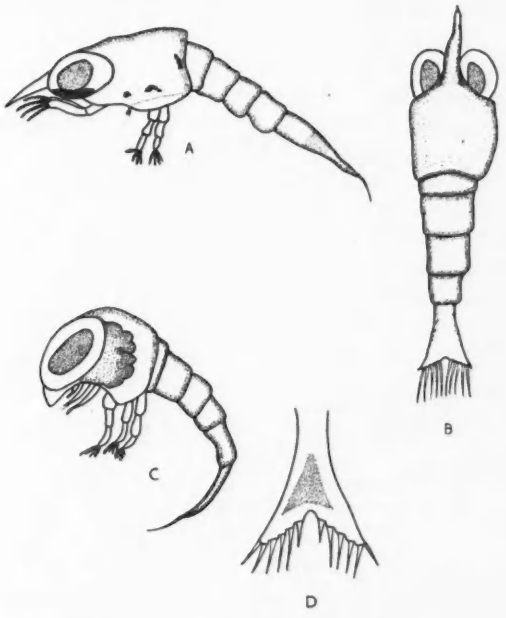

FIG. 17. A and B. Larvae of *Clibanarius tricolor*. C. Larva of *Calcinus tibicen*. D. Telson of same.

It occurs from below mean low water to nearly mean sea level on horizontal surfaces as well as under rocks and in tide pools. Breeding takes place from April to June. A typical zoea larva is released. It is 1.2 mm in length, has bright-green eyes, a brick-red gut which shows clearly through the exoskeleton, and a bright-blue patch on the tail.

Holothuria glaberrima Selenka (Fig. 18)

This species is found at all stations but is most abundant in areas exposed to wave action. It is abundant at Silver Sands, Conset Bay, and River Bay. It is a gregarious species and several dozen individuals may occur side by side. It varies in color from a warm brown to almost black.

It is a characteristic form of the surf zone and is rigidly restricted to the vertical limits of this zone. It lives firmly attached to the rock in holes and fissures with the densely branched tentacles opening and closing in response to wave action.

H. glaberrima breeds during June and July. The bright-pink eggs are 0.09 mm in diameter and develop to the two-celled stage within 2 hours of fertilization. A swimming blastula is released within 4 hours and a well-formed auricularia larva develops within 3 days.

At 3 days larvae reared in the laboratory were 1.3 mm long. No further growth occurred in the laboratory but at the end of 2 weeks a small percentage

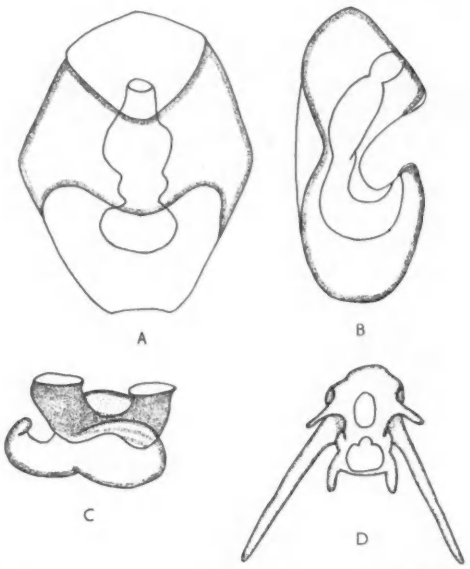

FIG. 18. A and B. Auricularia of *Holothuria glaberrima*. C. Metamorphosing larva of *Holothuria glaberrima*. D. Pluteus of *Echinometra lucunter*.

of larvae began to undergo metamorphosis. A few larvae developed adhesive pads and settled to the substrate. The larva shown in Fig. 18C is in the process of metamorphosis. It is 1.4 mm in length and has a pair of adhesive pads and a darkly pigmented body. The species thus has a fairly long pelagic life.

Echinometra lucunter (Linnaeus) (Fig. 18)

This common West Indian urchin is an extremely abundant species in Barbados. It occurs commonly below low water mark under flat rocks and on reef flats. It is found at all stations in the surf zone, in holes and fissures of the frontal platforms, or at low water mark at cliff stations. Most individuals live in more or less deep depressions which have been excavated in the rock.

Echinometra breeds during the summer from May to the end of August. The bright-orange eggs are 0.08 mm in diameter and are shed freely into the water. Plutei of this species occurred in the plankton throughout the summer and metamorphosing larvae were taken in plankton nets in July and August. Larvae were kept alive in the laboratory for a period of about 2 weeks. At the end of this period three pairs of pluteal arms had developed. The species thus has a fairly long pelagic life.

Phragmatopoma californica (Fewkes) (Fig. 19)

This sabellarid worm builds tubes of cemented sand grains. It occurs at River Bay, Bathsheba, and Conset Bay. Wherever present, colonies usually form a thick veneer over the rock surface.

It occurs from mean low water mark to about mean sea level. At River Bay it is localized in a small cave which is protected from wave action. At Bathsheba, where there are fairly extensive areas of the species, colonies occur from below mean low water to below mean sea level. At Conset Bay it is sparsely distributed in a narrow band at about mean low mark.

Breeding takes place in June and July. The eggs are blue-grey in color, 0.2 mm in diameter, and are carried in pouches at the bases of the parapodia. Freshly shed eggs are slightly sticky and adhere to one another and to the substrate. Fertilized eggs hatch in about 12 hours. The trochophore is 0.3 mm in diameter. It bears a fringe of cilia, a pair of long flagella, and is an active swimmer. Within 2 days the larva has a single chaetigerous segment and a pair of small red eyespots. Within a week it has developed four chaetigerous segments and is 2.2 mm long. A fairly long pelagic life is thus indicated for this species.

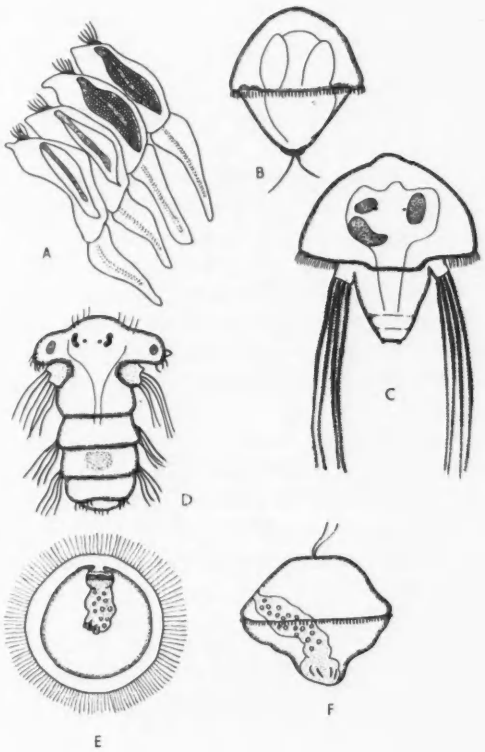

FIG. 19. A. Egg-bearing segments of female of *Phragmatopoma californica*. B. Trochophore of *Phragmatopoma californica*. C. Two-day-old larva of *Phragmatopoma californica*. D. Week-old larva of *Phragmatopoma californica*. E and F. Larvae of *Spirobranchus giganteus*.

Spirobranchus giganteus (Pallas) (Fig. 19)

This serpulid worm occurs in small numbers at all stations. It is exceedingly abundant at Silver Sands and at River Bay where it forms a covering over the pink zone and has even excluded colonization by the small vermetid *Spiroglyphus irregularis*. It occurs in small colonies at other stations within the limits of the pink zone. At Six Men's Bay a large colony has developed on the undersides of large boulders which are exposed to the air at low tide. Since no individuals are found on the upper surfaces of the boulders, some sort of preference for a shaded site is indicated.

Breeding takes place in the summer from May to the end of July. Eggs and sperm are shed freely into the water. The eggs are bright orange in color, 0.12 mm in diameter, and are sticky to touch. They hatch within a day of fertilization and an actively swimming trochophore is released. Since laboratory-reared specimens did not undergo any change or development within about a week of hatching, the species is presumed to have a fairly long pelagic life.

Bunodosoma kukenthali Pax (Fig. 20)

B. kukenthali is a brick-red anemone of moderate size. It is abundant at stations on the east and south coasts. At Silver Sands and Oistins it occurs in the surf zone in the cracks and fissures of the cliff below mean low water. At both these stations this zone is constantly wetted and the anemones are nearly always expanded. In this state they closely resemble in both form and color the echinoid *Echinometra lucunter* which lives in close proximity.

At River Bay, Bathsheba, and Conset Bay it is found in the pink zone on the frontal platforms. It occurs in holes in the rock as well as upon the surface. At Tent Bay it is found upon the larger boulders above mean sea level. Exposed to the air the species is normally contracted and is often dried and wrinkled in appearance.

Breeding occurs in the summer from June to August. The larvae are shed a few at a time and adhere to the bottom almost immediately. Freshly shed larvae are 0.36 mm in diameter. They are cream and pink in color with a few dark pigment spots and have a fringe of short cilia. Upon settling, the larvae may contract slightly and develop a thin cuticle with fine attachment strands.

Bunodosoma cavernata (Bosc) (Fig. 20)

B. cavernata is a handsomely colored anemone which occurs occasionally at all stations and is common on the east coast. It occurs chiefly in the surf zone and in tide pools or under rocks up to about mean sea level. At Bathsheba and Conset Bay it occurs in more sheltered sites.

The species breeds in June and July. The freshly shed larvae are 0.28 mm in diameter, light cream in color with scattered darker pigment granules, and are active swimmers. They are slightly sticky and settle within a day or so of hatching.

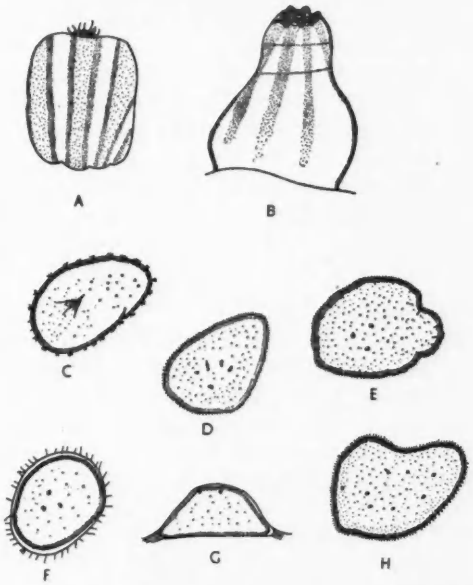

FIG. 20. A. Large swimming larva of *Bunodactis stelloides*. B. Settling larva of *Bunodactis stelloides*. C. Larva of *Phymanthus crucifer*. D and E. Larvae of *Bunodosoma cavernata*. F. Larva of *Bunodosoma kukenthali*. H. Small larva of *Bunodactis stelloides*.

Phymanthus crucifer Leseuer (Fig. 20)

Phymanthus is a beautifully colored anemone which is common only at Conset Bay. Here it lives on the frontal platform at about the mean low water mark and is exposed only during the period of lowest tides. It lies imbedded deep within fissures in the rock with the foot firmly attached and the oral face spread out in convolutions on the surface of the rock.

The larvae are released in July. They are free-swimming, 0.24 mm in diameter, and have tufts of cilia evenly distributed around the edge. They are pale yellow in color with scattered darker pigment granules. Settlement takes place within a day or two of their release.

Bunodactis stelloides (McMurrich) (Fig. 20)

This little anemone is quite common at all stations. It lives firmly attached to the substrate in cracks and fissures. The exposed oral disc collects and holds sand grains so that the animal is often completely concealed. It lives from below mean low water, under rocks and flat stones, to about mean sea level. It is abundant at River Bay in the pink zone, deeply imbedded in holes in the cliff face.

It is variable in color, varying from green to light red. Specimens exposed at low tide usually have their tentacles contracted so that only a small patch of sand grains is visible.

The species reproduces by budding and by the release of two types of larvae. Large actively swimming larvae were found in December and January, moving about in the body cavity and in the tentacles. They are barrel-shaped, light brown in color, and are 0.8 to 1.0 mm in length. They settled down on the bottom of culture dishes in the laboratory and began to develop tentacles 2–3 days after hatching.

The second type of larva was found in March and April. It is a much smaller form than the winter type and is irregular in shape. It is 0.3 mm, cream in color with red pigment spots, and is sticky to touch. Most larvae of this type settled down on the bottom within a few hours of their release.

Palythoa variabilis (Duerden)

This colonial zooanthid is found only at stations on the east coast, on the platforms fronting the cliff walls. It is abundant on the extensive rock flats at Bathsheba and Conset Bay in the region of mean low water. It shows a preference for shallow depressions and tide pools which hold water at low tide, although large colonies which are quite dry at low tide are common.

No breeding individuals were collected.

Zooanthus pulchellus (Duchassaing and Michelotti)

This species occurs at Six Men's Bay where it is found on a horizontal surface just below mean low water mark and on the cliff walls at Conset Bay at somewhat higher levels. Large colonies occur at both stations.

No larval stages were collected from this species.

Vertebrates

Two species of small fish occur commonly at most stations. They are a small goby with a ventral sucker (family Gobiidae) and a small clingfish (family Gobeisocidae).

The goby occurs commonly at all stations, in tide pools on rocky platforms and often upon the vertical face of the cliffs themselves. Its vertical movement coincides with the tide level. The young of this species appear in large numbers on the shores in August.

The clingfish is common at Bathsheba and under rocks on the boulder beach at Tent Bay. Here it occurs up to a size of 2 in. in length. It is common in the surf zone of the rocky platform at Six Men's where it lives closely associated with the sea urchin *Echinometra lucunter*, attached firmly to the bottom of the depressions in which the urchins live. These specimens are invariably small, darkly pigmented, and several individuals may be found together.

Reproductive and Larval Ecology

In addition to the usual problems of dispersal and larval settlement which confront all littoral animals there are other factors which influence the capacity for establishment of intertidal forms. The relative advantages of different modes of fertilization of marine bottom invertebrates has been outlined by Thorson (13). He has pointed out that copulation is the most certain method of

fertilization and least wasteful of the larval stock. For intertidal animals this method of reproduction has an especial significance.

If fertilization is external—eggs and sperm shed freely into the water—and is to be successful, several conditions must be met. First, the adults must all be sexually mature at approximately the same time. Many of the species dealt with in this study breed continuously throughout the year or have a breeding season lasting over a period of several months. If a population of animals is to become sexually mature at the same time, a short breeding season is implied. Thus this method of fertilization cannot be an advantageous one but wasteful to the larval stock of the group under consideration.

Forms which live above the high water mark must also move downwards into the water at sexual maturity if the eggs and sperm are to be freely shed. If copulation takes place there would be no need for animals living at the upper levels in the intertidal zone to migrate downwards, breeding aggregations would be less important, and the population would not need to be sexually mature at the same time.

The copulators and species with external fertilization for the animals whose method of reproduction has been here described are listed below.

Copulators	External Fertilization
Thais patula	*Fissurella barbadensis*
Thais floridana	*Acmaea jamaicensis*
Thais deltoidea	*Hemitoma octoradiata*
Littorina maleagris	*Acanthopleura granulata*
Littorina ziczac	*Chiton marmoratus*
Tectarius tuberculatus	*Chiton tuberculatus*
Tectarius muricatus	*Tegula excavata*
Neritina pupa	*Isognomon listeri*
Nerita peleronta	*Echinometra lucunter*
Nerita versicolor	*Holothuria glaberrima*
Nerita tessellata	*Phragmatopoma californica*
Conus mus	*Spirobranchus giganteus*
Leucozonia ocellata	
Planaxis nucleus	
Planaxis lineatus	
Spiroglyphus irregularis	
Petaloconchus varians	
Astrea caelata (pseudo)	
Lithotrya dorsalis	
Tetraclita squamosa	
Grapsus grapsus	
Petrolisthes vanderhorsti	
Eriphia gonogra	
Clibanarius tricolor	
Calcinus tibicen	

Thus, of 37 species 25 are copulators and only 12 have external fertilization.

Intertidal forms have a limited surface area in which they can settle and develop to maturity when compared to bottom communities. Larvae with a long pelagic life may thus find themselves beyond reach of the shores upon which they must settle. A short pelagic life would be of advantage to intertidal forms in limiting dispersal and this is especially true in the case of an island such as Barbados, lying at some distance from any large land mass.

The problem of limiting the dispersal of larvae can be solved in two ways: by development of eggs within a capsule laid in the intertidal zone, or by

brood protection, and the settlement of larvae immediately the eggs are hatched. Listed below are the species which produce egg capsules or have an extremely short pelagic life.

Egg Capsules	Short Pelagic Life
Thais patula	*Fissurella barbadensis*
Thais floridana	*Acmaea jamaicensis*
Thais deltoidea	*Hemitoma octoradiata*
Nerita peleronta	*Acanthopleura granulata*
Nerita versicolor	*Chiton marmoratus*
Nerita tessellata	*Chiton tuberculatus*
Tectarius tuberculatus	*Leucozonia ocellata*
Tectarius muricatus	*Bunodosoma cavernata*
Littorina ziczac	*Bunodosoma kukenthali*
Littorina maleagris	*Phymanthus crucifer*
Neritina pupa	*Bunodactis stelloides*
Conus mus	*Spiroglyphus irregularis*
Leucozonia ocellata	

Of a total of 42 species whose early larval stages are here described, 25 produce egg capsules or limit their dispersal by means of a short larval life. Of the remainder most are found at low levels in the intertidal region and some are littoral as well as intertidal. The littoral forms are as follows: *Echinometra lucunter*, *Holothuria glaberrima*, *Eriphia gonogra*, *Calcinus tibicen*, *Clibanarius tricolor*, *Petrolisthes vanderhorsti*, *Isognomon listeri*, and the two species of fish. The area of settlement for these species is widely increased and for all species except *Isognomon* migration into the intertidal region is possible.

Only five species which are truly intertidal produce larvae which do not possess the adaption of a shortened pelagic life by one means or another. They are the two barnacles *Lithotrya dorsalis* and *Tetraclita squamosa*, the vermetid *Petaloconchus varians*, and the two worms *Spirobranchus giganteus* and *Phragmatopoma californica*. Of these, however, the first three are ovoviviparous.

There is, then, a tendency for the following adaptions in the reproduction and larval history of intertidal forms in this study: the adults are copulators, they are ovoviviparous, or produce egg capsules, and the larvae tend to have a much shortened free pelagic life. Through these adaptions restriction of dispersal is achieved.

The vermetid *Spiroglyphus irregularis* is an excellent example of a form well adapted in the early stages of its life history to life in the intertidal region. Its abundance at all stations is a measure of its success.

The species breeds throughout the year and is ovoviviparous. The larvae settle immediately the eggs are hatched and become firmly established within a few hours. The loss of the larval stock is thus kept at a minimum, for the young remain within the space limit of the adult environment.

There is a danger in this, however, for the dispersal of the species to other suitable environments is sharply decreased. This problem has been nicely solved by the additional production of egg capsules which are released freely into the water. The eggs develop within the capsules and depending upon the developmental stage at which they are released may take up to 2 weeks before hatching. A swimming larva, which settles within a few days, is released.

The pelagic existence of the species is thus spent within the egg capsule but is nonetheless effective as a means of species dispersal.

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I have to thank Dr. Gilbert Voss of the Marine Laboratory, University of Miami, Florida, and Dr. Harald A. Rehder of the U.S. National Museum for their identifications of molluscs; Dr. Fenner Chace, Jr., of the U.S. National Museum for his identification of crabs; Dr. Joan Marsden of McGill University, Montreal, for her identification of annelids; Dr. Charles E. Cutress of the U.S. National Museum for his identification of anemones; and Dr. Jacques Zanaveld of the Caribbean Marine Biological Institute, Curacao, for the identification of a collection of algae.

Thanks are also due to Dr. J. E. Dinger and Mr. Ben. G. Julian of the U.S. Office of Naval Research for permission to use wave records taken from installations on Barbados and to Dr. William L. Donn of the Lamont Geological Observatory, New York, for making tide data available.

References

1. Doty, M. S. Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific Coast. Ecology, **27**(4), 315–328 (1946).
2. Doty, M. S. Rocky intertidal surfaces. Geol. Soc. Am. Mem. **67** (1), 535–585 (1957).
3. Doty, M. S. and Archer, J. G. An experimental test of the tide factor hypothesis. Am. J. Botany, **37**(6), 458–464 (1950).
4. Kitching, J. A. Studies in sublittoral ecology. II. Recolonization at the upper margin of the sublittoral region; with a note on the denudation of a *Laminaria* forest by storms. J. Ecol. **25**, 482–495 (1937).
5. Moore, H. B. The colonization of a new rocky shore at Plymouth. J. Animal Ecol. **8**(1), 29–38 (1939).
6. Senn, A. Paleogene of Barbados and its bearing on history and structure of the Antillean–Caribbean region. Bull. Am. Assoc. Petrol. Geologists, **24**(9) (1940).
7. Smith, F. G. W. Effect of water current upon the attachment and growth of barnacles. Biol. Bull. **90**(1), 51–70 (1946).
8. Smith, F. G. W. Surface illumination and barnacle attachment. Biol. Bull. **94**(1), 33–39 (1948).
9. Southward, A. J. Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution. J. Marine Biol. Assoc. United Kingdom, **37**(1), 49–66 (1958).
10. Stephenson, T. A. and Stephenson, A. The universal features of zonation between tide-marks on rocky coasts. J. Ecol. **37**(2), 289–305 (1949).
11. Stephenson, T. A. and Stephenson, A. Life between tide-marks in North America. I. The Florida keys. J. Ecol. **38**(2), 354–402 (1950).
12. Stephenson, T. A. and Stephenson, A. Life between tide-marks in North America. II. Northern Florida and the Carolinas. J. Ecol. **40**(1), 1–49 (1952).
13. Thorson, G. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the sound (øresund). Medd. Komm. Danmarks fiskeri-og Havundersøgelser. Ser. Plankton, **4**(1), 1–523 (1946).
14. Wilson, D. P. The influence of the nature of the substratum on the metamorphosis of the larvae of marine animals, especially the larvae of *Ophelia bicornis* Savigny. Ann. inst. océanogr. Paris **27**(2), 49–156 (1952).
15. Sailing Directions for the West Indies. 2. The Lesser Antilles and the coast of Venezuela. Publ. 129, U.S. Navy Hydro Office, Washington 25, D.C. 1948.

NOTES

NOTE ON THE USE OF McILVAINE'S BUFFER SOLUTIONS IN STAINING BLOOD PROTOZOA

G. LUBINSKY

When using Romanowsky-type stains in the field one is sometimes obliged to resort to buffers other than Sörensen's. An incidental absence of monobasic sodium phosphate has forced the present writer more than once to replace it by citric acid, thus converting Sörensen's buffers into McIlvaine's.

A 2% solution of citric acid and a 3% solution of anhydrous disodium phosphate were mixed to obtain 20 cc of the buffer. Figure 1 shows the volumes mixed for the preparation of a buffer of a certain pH. When used for staining purposes these buffers have had to be diluted 1:4 with distilled water.

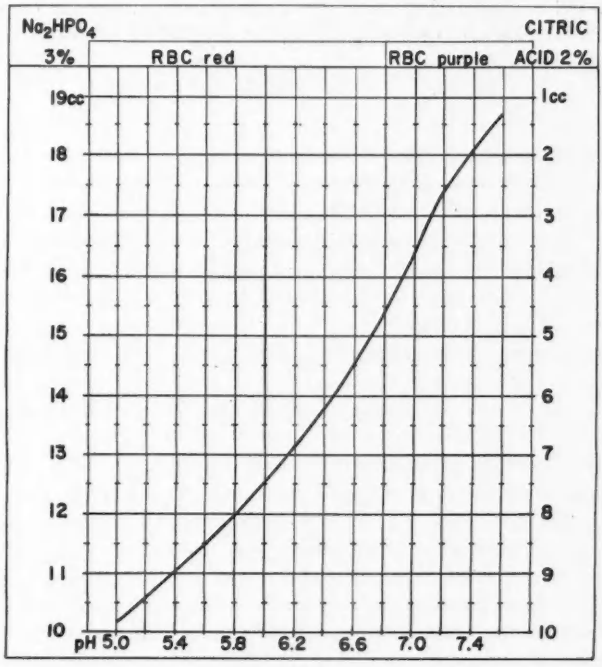

FIG. 1

A buffer of pH 6.7 was also made in mixing equal parts of 2% citric acid with 10% disodium phosphate. One drop of the mixture was added to each milliliter of distilled water used for the dilution of strains.

When phosphates were not available a buffer of pH 6.8 was prepared by dissolving 18 g of sodium citrate and 0.3 g of citric acid in 1000 cc of distilled water and used undiluted. Both the McIlvaine's and the citrate buffers were used with Wright's, Giemsa's, and May-Grünwald stains. The results were not inferior to those obtained with the usual phosphate buffer solutions.

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A NOTE ON AN UNIDENTIFIED MICROSPORIDIAN ASSOCIATED WITH ALETIA OXYGALA LUTEOPALLENS (SM.) (LEPIDOPTERA:NOCTUIDAE)[1]

H. M. THOMSON[2] AND W. SMIRNOFF[3]

Larvae of *Aletia oxygala luteopallens* were found dead and dying near Quebec City, Quebec. Dark field examination of these larvae revealed the presence of a polyhedron virus and a protozoon in the mid-gut epithelia. Unfortunately, the source of the infected material disappeared before the investigations were complete and sufficient data were not acquired to allow specific identification of the protozoon. However, the life cycle of this organism has been determined and is described in this note.

The earliest recognizable stages of the protozoon are binucleate schizonts measuring about 2 μ in diameter (Fig. 1, *a*). The cytoplasm is evenly stained. Sometimes a light-staining area appears around the nuclei, which may have clear centers. The schizonts grow and multiply (Fig. 1, *b*, *c*, *d*, *e*). The largest schizonts may contain as many as 12 nuclei arranged in pairs (Fig. 1, *f*). It would appear that the paired nuclei unite and the schizonts break up into uninucleate meronts (Fig. 1, *g*, *h*). The spore-forming stages are initiated by multiplication of the nuclei. The sporonts thus formed may contain as few as 4 or as many as 60 or more nuclei. The nuclei are very compact and darkly stained. The sporont is generally spherical with vacuolated cytoplasm and may measure 10 μ in diameter (Fig. 1, *i*, *j*). It is believed that the nuclei unite in pairs as the number of nuclei within a sporont greatly exceeds the number of

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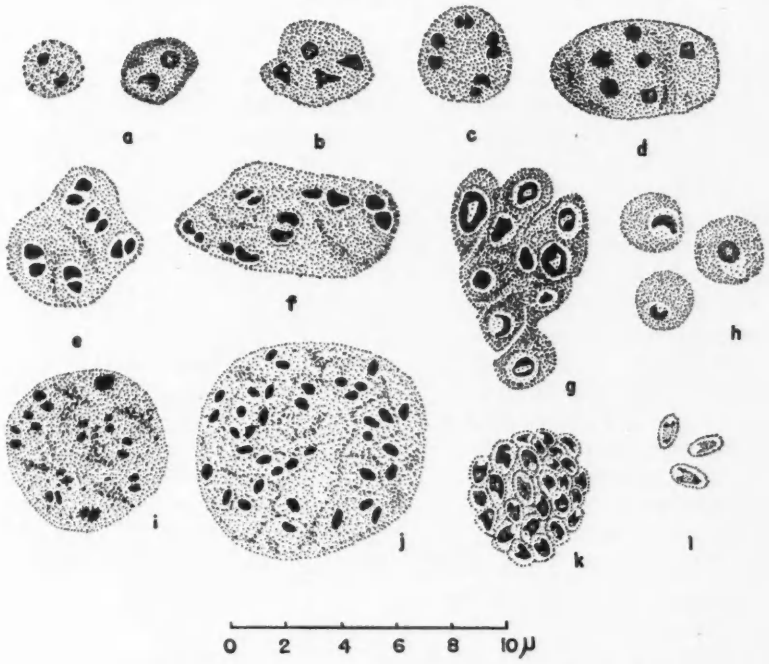

FIG. 1. Stages in the life cycle of a microsporidian parasite of *A. luteopallens*: *a–f*, schizonts; *g*, schizogony; *h*, meronts; *i–j*, sporonts; *k*, pansporoblast; *l*, spores.

sporoblasts eventually produced. A spore wall encloses each pair of nuclei (Fig. 1, *k*) forming the sporoblasts. Within the sporoblasts the cytoplasm takes on a girdle-like shape leaving a vacuole at one end. The nuclei no longer stain distinctly. The fully developed pansporoblast may attain a diameter of 6 μ (Fig. 1, *k*). Mature stained spores measure 0.75×1.5 μ (Fig. 1, *l*).

As the sporonts develop into a variable number of spores, often more than 16, the organism is a member of the genus *Plistophora* Gurley (Kudo 1946). As was mentioned in the introduction, the investigation of this organism was not complete and it was not possible to carry the identification beyond the genus level.

1. KUDO, R. R. Protozoology. 3rd ed. Chas. C. Thomas, Publisher, Springfield, Illinois, U.S.A. 1946.

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A NOTE ON SOME HELMINTHS FROM MALAYAN ANIMALS

B. J. MYERS

During a survey to determine the significance of Malayan animals as a reservoir for *Wuchereria malayi* undertaken by the Institute for Medical Research, Kuala Lumpur, intestinal helminths were collected by Professor A. A. Sandosham of the University of Malaya and it was due to his kindness that the present material was made available for study.

House shrews (*Suncus murinus*) were collected in Singapore by the Singapore City Council for Dr. Marshall Laird.

The distribution of the host is shown as follows, immediately after the name:

Ancylostoma ceylanicum Looss, 1911
Callosciurus caniceps (M)*
Callosciurus notatus (M)

Ancylostoma sp. (females only)
Callosciurus caniceps (M)
Callosciurus notatus (M)
Callosciurus tenuis (M)
Suncus murinus (S)*

Filaroidea sp. (fragments)
Brown squirrel (M)
Tupaia glis (M)
Nannosciurus sp. (B)*

Gongylonema orientale Yokagawa, 1924
Callosciurus notatus (M)

Gongylonema sp. (females only)
Crocidura sp. (M)

Longistriata cristata (Gedoelst, 1917) Travassos and Darriba, 1929 nec. Cameron, 1939
Callosciurus caniceps (M)
Callosciurus nigrovittatus (M)
Callosciurus notatus (M)
Callosciurus prevosti (B)
Tupaia montana (M)

Physaloptera sp. (females only)
Callosciurus nigrovittatus (M)
Callosciurus notatus (M)

Physaloptera larvae
Callosciurus notatus (M)

*(M) Malaya; (B) North Borneo; (S) Singapore.

Rictularia sp. (females only)
Tupaia montana (M)

Spirura talpae (Gmelin, 1790) Blanchard, 1849
Tupaia glis (M)

Spirura sp. (females only)
Tupaia glis (M)

Subulura pigmentata Gedoelst, 1917
Callosciurus caniceps (M)
Callosciurus prevosti (B)
Tupaia glis (M)

Subulura sp. (females only)
Callosciurus tenuis (M)

Trichuris sp. (females only)
Callosciurus prevosti (B)

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NOTES TO CONTRIBUTORS

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